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(54) Title: TOTIPOTENT HEMATOPOIETIC STEM CELL RECEPTORS AND THEIR LIGANDS

(57) Abstract

Isolated mammalian nucleic acid molecules encoding receptor protein tyrosine kinases expressed in primitive hematopoietic cells and not expressed in mature hematopoietic cells are provided. Also included are the receptors encoded by such nucleic acid molecules; the nucleic acid molecules encoding receptor protein tyrosine kinases having the sequences shown in Figure 1a (murine Flk2), Figure 1b (human Flk2) and Figure 2 (murine Flk1); the receptor protein tyrosine kinases having the amino acid sequences shown in Figure 1a, Figure 1b and Figure 2; ligands for the receptors; nucleic acids sequences that encode the ligands; and methods of stimulating the proliferation and/or differentiation of primitive mammalian hematopoietic stem cells comprising contacting the stem cells with a ligand that binds to a receptor protein tyrosine kinase expressed in primitive mammalian hematopoietic cells and not expressed in mature hematopoietic cells.

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**TOTIPOTENT HEMATOPOIETIC STEM CELL
RECEPTORS AND THEIR LIGANDS**

This application is a continuation-in-part of serial number 08/125,669, filed September 23, 1993, which is a continuation-in-part of serial number 08/096,759, filed July 22, 1993, which is a continuation-in-part of serial number 08/081,508, filed June 21, 1993, which is a continuation-in-part of serial number 08/080,244, filed June 18, 1993, which is a continuation-in-part of serial number 08/076,022, filed June 9, 1993, which is a continuation-in-part of serial number 08/045,272, filed April 1, 1993, which is a continuation-in-part of serial number 08/005,941, filed January 15, 1993, which is a continuation-in-part of serial number 07/977,451, filed November 19, 1992, which is a continuation-in-part of serial number 07/975,049 filed November 12, 1992, which is a continuation-in-part of serial number 07/906,397 filed June 26, 1992 which is a continuation-in-part of serial number 07/813,593 filed December 24, 1991, which is a continuation-in-part of serial number 07/793,065 filed November 15, 1991, which is a continuation-in-part of serial number 07/728,913 filed June 28, 1991, which is a continuation-in-part of serial number 07/679,666 filed April 2, 1991, all of which are incorporated herein by reference.

The invention described in this application was made with U.S. government support from Grant Numbers R01-CA45339 and R01-DK42989 awarded by the National Institutes of Health. The government has certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates to hematopoietic stem cell receptors, ligands for such receptors, and nucleic acid molecules encoding such receptors and ligands.

BACKGROUND OF THE INVENTION

5 The mammalian hematopoietic system comprises red and white blood cells. These cells are the mature cells that result from more primitive lineage-restricted cells. The cells of the hematopoietic system have been reviewed by Dexter and Spooncer in the Annual Review of Cell Biology 3, 423-441 (1987).

10 The red blood cells, or erythrocytes, result from primitive cells referred to by Dexter and Spooncer as erythroid burst-forming units (BFU-E). The immediate progeny of the erythroid burst-forming units are called erythroid colony-forming units (CFU-E).

15 The white blood cells contain the mature cells of the lymphoid and myeloid systems. The lymphoid cells include B lymphocytes and T lymphocytes. The B and T lymphocytes result from earlier progenitor cells referred to by Dexter and Spooncer as preT and preB cells.

20 The myeloid system comprises a number of cells including granulocytes, platelets, monocytes, macrophages, and megakaryocytes. The granulocytes are further divided into neutrophils, eosinophils, basophils and mast cells.

25 Each of the mature hematopoietic cells are specialized for specific functions. For example, erythrocytes are responsible for oxygen and carbon dioxide transport. T and B lymphocytes are responsible for cell-and antibody-mediated immune responses, respectively. Platelets are involved in blood clotting. Granulocytes and macrophages act generally as scavengers and accessory cells in the immune response against invading organisms and their by-products.

35 At the center of the hematopoietic system lie one or more

totipotent hematopoietic stem cells, which undergo a series of differentiation steps leading to increasingly lineage-restricted progenitor cells. The more mature progenitor cells are restricted to producing one or two lineages. Some examples of lineage-restricted progenitor cells mentioned by Dexter and Spooner include granulocyte/macrophage colony-forming cells (GM-CFC), megakaryocyte colony-forming cells (Meg-CFC), eosinophil colony-forming cells (Eos-CFC), and basophil colony-forming cells (Bas-CFC). Other examples of progenitor cells are discussed above.

The hematopoietic system functions by means of a precisely controlled production of the various mature lineages. The totipotent stem cell possesses the ability both to self renew and to differentiate into committed progenitors for all hematopoietic lineages. These most primitive of hematopoietic cells are both necessary and sufficient for the complete and permanent hematopoietic reconstitution of a radiation-ablated hematopoietic system in mammals. The ability of stem cells to reconstitute the entire hematopoietic system is the basis of bone marrow transplant therapy.

It is known that growth factors play an important role in the development and operation of the mammalian hematopoietic system. The role of growth factors is complex, however, and not well understood at the present time. One reason for the uncertainty is that much of what is known about hematopoietic growth factors results from in vitro experiments. Such experiments do not necessarily reflect in vivo realities.

In addition, in vitro hematopoiesis can be established in the absence of added growth factors, provided that marrow stromal cells are added to the medium. The relationship between stromal cells and hematopoietic growth factors in vivo is not understood. Nevertheless, hematopoietic growth factors have been shown to be

highly active in vivo.

From what is known about them, hematopoietic growth factors appear to exhibit a spectrum of activities. At one end of the spectrum are growth factors such as erythropoietin, which is believed to promote proliferation only of mature erythroid progenitor cells. In the middle of the spectrum are growth factors such as IL-3, which is believed to facilitate the growth and development of early stem cells as well as of numerous progenitor cells. Some examples of progenitor cells induced by IL-3 include those restricted to the granulocyte/macrophage, eosinophil, megakaryocyte, erythroid and mast cell lineages.

At the other end of the spectrum is the hematopoietic growth factor that, along with the corresponding receptor, was discussed in a series of articles in the October 5, 1990 edition of Cell. The receptor is the product of the W locus, c-kit, which is a member of the class of receptor protein tyrosine kinases. The ligand for c-kit, which is referred to by various names such as stem cell factor (SCF) and mast cell growth factor (MGF), is believed to be essential for the development of early hematopoietic stem cells and cells restricted to the erythroid and mast cell lineages in mice; see, for example, Copeland et al., Cell 63, 175-183 (1990).

25

It appears, therefore, that there are growth factors that exclusively affect mature cells. There also appear to be growth factors that affect both mature cells and stem cells. The growth factors that affect both types of cells may affect a small number or a large number of mature cells.

30

There further appears to be an inverse relationship between the ability of a growth factor to affect mature cells and the ability of the growth factor to affect stem cells. For example, the c-kit ligand, which stimulates a small number of mature

35

cells, is believed to be more important in the renewal and development of stem cells than is IL-3, which is reported to stimulate proliferation of many mature cells (see above).

5 Prior to the present specification, there have been no reports of growth factors that exclusively stimulate stem cells in the absence of an effect on mature cells. The discovery of such growth factors would be of particular significance.

10 As mentioned above, c-kit is a protein tyrosine kinase (pTK). It is becoming increasingly apparent that the protein tyrosine kinases play an important role as cellular receptors for hematopoietic growth factors. Other receptor pTKs include the receptors of colony stimulating factor 1 (CSF-1) and PDGF.

15 The pTK family can be recognized by the presence of several conserved amino acid regions in the catalytic domain. These conserved regions are summarized by Hanks et al. in Science 241, 42-52 (1988), see Figure 1 starting on page 46 and by Wilks in
20 Proc. Natl. Acad. Sci. USA 86, 1603-1607 (1989), see Figure 2 on page 1605.

25 Additional protein tyrosine kinases that represent hematopoietic growth factor receptors are needed in order more effectively to stimulate the self-renewal of the totipotent hematopoietic stem cell and to stimulate the development of all cells of the hematopoietic system both in vitro and in vivo. Novel hematopoietic growth factor receptors that are present only on primitive stem cells, but are not present on mature progenitor
30 cells, are particularly desired. Ligands for the novel receptors are also desirable to act as hematopoietic growth factors. Nucleic acid sequences encoding the receptors and ligands are needed to produce recombinant receptors and ligands.

SUMMARY OF THE INVENTION

These and other objectives as will be apparent to those with ordinary skill in the art have been met by providing isolated mammalian nucleic acid molecules encoding receptor protein tyrosine kinases expressed in primitive hematopoietic cells and not expressed in mature hematopoietic cells. Also included are the receptors encoded by such nucleic acid molecules; the nucleic acid molecules encoding receptor protein tyrosine kinases having the sequences shown in Figure 1a.1-1a.6 (hereinafter Figure 1a)(murine Flk2), Figure 1b.1-1b.6 (hereinafter Figure 1b)(human Flk2) and Figure 2.1-2.9 (hereinafter Figure 2)(murine Flk1)(See SEQ. ID. NOS. 1, 3 and 5, respectively); the receptor protein tyrosine kinases having the amino acid sequences shown in Figure 1a, Figure 1b and Figure 2 (See SEQ. ID. NOS. 2, 4 and 6, respectively); ligands for the receptors; nucleic acid sequences that encode the ligands; and methods of stimulating the proliferation of primitive mammalian hematopoietic stem cells comprising contacting the stem cells with a ligand that binds to a receptor protein tyrosine kinase expressed in primitive mammalian hematopoietic cells and not expressed in mature hematopoietic cells.

DESCRIPTION OF THE FIGURES

Figure 1a.1 through 1a.6 shows the cDNA and amino acid sequences of murine Flk2. All subsequent references to Figure 1a are intended to refer to Figure 1a.1 through 1a.6. The amino acid residues occur directly below the nucleotides in the open reading frame. Amino acids -27 to -1 constitute the hydrophobic leader sequence. Amino acids 1 to 517 constitute the extracellular receptor domain. Amino acids 518 to 537 constitute the transmembrane region. Amino acids 538 to 966 constitute the intracellular catalytic domain. Counting amino acid residue -27 as residue number 1, the following amino acid residues in the

intracellular domain are catalytic sub-domains identified by Hanks (see above): 618-623, 811-819, 832-834, 857-862, 872-878. The sequence at residues 709-785 is a signature sequence characteristic of Flk2. The protein tyrosine kinases generally have a signature sequence in this region. (See SEQ. ID. NOS. 1-2)

Figure 1b.1 through 1b.6 shows the complete cDNA and amino acid sequences of human Flk2 receptor. All subsequent references to Figure 1b are intended to refer to Figure 1b.1 through 1b.6. Amino acids -27 to -1 constitute the hydrophobic leader sequence. Amino acids 1 to 516 constitute the extracellular receptor domain. Amino acids 517 to 536 constitute the transmembrane region. Amino acids 537 to 966 constitute the intracellular catalytic domain. (See SEQ. ID. NOS. 3-4)

Figure 2.1 through 2.9 shows the cDNA and amino acid sequences of murine Flk1. All subsequent references to Figure 2 are intended to refer to Figure 2.1 through 2.9. Amino acids -19 to -1 constitute the hydrophobic leader sequence. Amino acids 1 to 743 constitute the extracellular receptor domain. Amino acids 744 to 765 constitute the transmembrane region. Amino acids 766 to 1348 constitute the intracellular catalytic domain. (See SEQ. ID. NOS. 5-6)

Figure 3 shows the time response of binding between a murine stromal cell line (2018) and APTag-Flk2 as well as APTag-Flk1. APTag without receptor (SEAP) is used as a control. See Example 8.

Figure 4 shows the dose response of binding between stromal cells (2018) and APTag-Flk2 as well as APTag-Flk1. APTag without receptor (SEAP) is used as a control. See Example 8.

DETAILED DESCRIPTION OF THE INVENTIONReceptors

5 In one embodiment, the invention relates to an isolated mammalian nucleic acid molecule encoding a receptor protein tyrosine kinase expressed in primitive mammalian hematopoietic cells and not expressed in mature hematopoietic cells.

10 The nucleic acid molecule may be a DNA, cDNA, or RNA molecule. The mammal in which the nucleic acid molecule exists may be any mammal, such as a mouse, rat, rabbit, or human.

15 The nucleic acid molecule encodes a protein tyrosine kinase (pTK). Members of the pTK family can be recognized by the conserved amino acid regions in the catalytic domains. Examples of pTK consensus sequences have been provided by Hanks et al. in Science 241, 42-52 (1988); see especially Figure 1 starting on page 46 and by Wilks in Proc. Natl. Acad. Sci. USA 86, 1603-1607
20 (1989); see especially Figure 2 on page 1605. A methionine residue at position 205 in the conserved sequence WMAPES is characteristic of pTK's that are receptors.

25 The Hanks et al article identifies eleven catalytic subdomains containing pTK consensus residues and sequences. The pTKs of the present invention will have most or all of these consensus residues and sequences.

30 Some particularly strongly conserved residues and sequences are shown in Table 1.

TABLE 1

Conserved Residues and Sequences in pTKs¹

<u>Position</u> ²	<u>Residue or Sequence</u>	<u>Catalytic Domain</u>
------------------------------	----------------------------	-------------------------

	50	G	I
	52	G	I
	57	V	I
	70	A	II
5	72	K	II
	91	E	III
	166	D	VI
	171	N	VI
	184-186	DFG	VII
10	208	E	VIII
	220	D	IX
	225	G	IX
	280	R	XI

- 15 1. See Hanks et al., Science 241, 42-52 (1988)
 2. Adjusted in accordance with Hanks et al., Id.

20 A pTK of the invention may contain all thirteen of these highly conserved residues and sequences. As a result of natural or synthetic mutations, the pTKs of the invention may contain fewer than all thirteen strongly conserved residues and sequences, such as 11, 9, or 7 such sequences.

25 The receptors of the invention generally belong to the same class of pTK sequences that c-kit belongs to. It has surprisingly been discovered, however, that a new functional class of receptor pTKs exists. The new functional class of receptor pTKs is expressed in primitive hematopoietic cells, but not expressed in mature
 30 hematopoietic cells.

For the purpose of this specification, a primitive hematopoietic cell is totipotent, i.e. capable of reconstituting all hematopoietic blood cells in vivo. A mature hematopoietic
 35 cell is non-self-renewing, and has limited proliferative capacity - i.e., a limited ability to give rise to multiple lineages. Mature hematopoietic cells, for the purposes of this specification, are generally capable of giving rise to only one or two lineages in vitro or in vivo.

40

It should be understood that the hematopoietic system is complex, and contains many intermediate cells between the primitive totipotent hematopoietic stem cell and the totally committed mature hematopoietic cells defined above. As the stem cell develops into increasingly mature, lineage-restricted cells, it gradually loses its capacity for self-renewal.

The receptors of the present invention may and may not be expressed in these intermediate cells. The necessary and sufficient condition that defines members of the new class of receptors is that they are present in the primitive, totipotent stem cell or cells, and not in mature cells restricted only to one or, at most, two lineages.

An example of a member of the new class of receptor pTKs is called fetal liver kinase 2 (Flk2) after the organ in which it was found. There is approximately 1 totipotent stem cell per 10^4 cells in mid-gestation (day 14) fetal liver in mice. In addition to fetal liver, Flk2 is also expressed in fetal spleen, fetal thymus, adult brain, and adult marrow.

For example, Flk2 is expressed in individual multipotential CFU-Blast colonies capable of generating numerous multilineage colonies upon replating. It is likely, therefore, that Flk2 is expressed in the entire primitive (i.e. self-renewing) portion of the hematopoietic hierarchy. This discovery is consistent with Flk2 being important in transducing putative self-renewal signals from the environment.

It is particularly relevant that the expression of Flk2 mRNA occurs in the most primitive thymocyte subset. Even in two closely linked immature subsets that differ in expression of the IL-2 receptor, Flk2 expression segregates to the more primitive subset lacking an IL-2 receptor. The earliest thymocyte subset is believed to be uncommitted. Therefore, the thymocytes

expressing Flk2 may be multipotential. Flk2 is the first receptor tyrosine kinase known to be expressed in the T-lymphoid lineage.

5 The fetal liver mRNA migrates relative to 28S and 18S ribosomal bands on formaldehyde agarose gels at approximately 3.5 kb, while the brain message is considerably larger. In adult tissues, Flk2 m-RNA from both brain and bone marrow migrated at approximately 3.5 kb.

10 A second pTK receptor is also included in the present invention. This second receptor, which is called fetal liver kinase 1 (Flk1), is not a member of the same class of receptors as Flk2, since Flk1 may be found in some more mature
15 hematopoietic cells. The amino acid sequence of murine Flk1 is given in Figure 2. (See SEQ. ID. NOS. 5-6)

20 The present invention includes the Flk1 receptor as well as DNA, cDNA and RNA encoding Flk1. The DNA sequence of murine Flk1 is also given in Figure 2. (See SEQ. ID. NO. 5) Flk1 may be found in the same organs as Flk2, as well as in fetal brain, stomach, kidney, lung, heart and intestine; and in adult kidney, heart, spleen, lung, muscle, and lymph nodes.

25 The receptor protein tyrosine kinases of the invention are known to be divided into easily found domains. The DNA sequence corresponding to the pTKs encode, starting at their 5'-ends, a hydrophobic leader sequence followed by a hydrophilic extracellular domain, which binds to, and is activated by, a
30 specific ligand. Immediately downstream from the extracellular receptor domain, is a hydrophobic transmembrane region. The transmembrane region is immediately followed by a basic catalytic domain, which may easily be identified by reference to the Hanks et al. and Wilks articles discussed above.

The following table shows the nucleic acid and amino acid numbers that correspond to the signal peptide, the extracellular domain, the transmembrane region and the intracellular domain for murine Flk1 (mFlk1), murine Flk2 (mFlk2) and human Flk2 (hFlk2).

5

mFlk1

	<u>Signal Peptide</u>	<u>Extracellular</u>	<u>Transmembrane</u>	<u>Intracellular</u>
aa #	-19 to -1	1 to 743	744 to 765	766 to 1348
aa code	M A	A E	V V	R A
na #	208-264	265-2493	2494-2559	2560-4308

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mFlk2

	<u>Signal Peptide</u>	<u>Extracellular</u>	<u>Transmembrane</u>	<u>Intracellular</u>
aa #	-27 to -1	1 to 517	518 to 537	538 to 966
aa code	M T	N S	F C	H S
na #	31-111	112-1662	1663-1722	1723-3006

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hFlk2

	<u>Signal Peptide</u>	<u>Extracellular</u>	<u>Transmembrane</u>	<u>Intracellular</u>
aa #	-27 to -1	1 to 516	517 to 536	537 to 966
aa code	M N	Q F	Y C	H S
na #	58-138	139-1689	1690-1746	1747-3036

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The present invention includes the extracellular receptor domain lacking the transmembrane region and catalytic domain. Preferably, the hydrophobic leader sequence is also removed from the extracellular domain. In the case of human and murine Flk2, the hydrophobic leader sequence includes amino acids -27 to -1. (See SEQ. ID. NOS. 2 and 4)

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These regions and domains may easily be visually identified by those having ordinary skill in the art by reviewing the amino acid sequence in a suspected pTK and comparing it to known pTKs. For example, referring to Figure 1a, the transmembrane region of Flk2, which separates the extracellular receptor domain from the

catalytic domain, is encoded by nucleotides 1663 (T) to 1722 (C). These nucleotides correspond to amino acid residues 545 (Phe) to 564 (Cys). (See SEQ. ID. NOS. 1-2) The amino acid sequence between the transmembrane region and the catalytic sub-domain (amino acids 618-623) identified by Hanks et al. as sub-domain I (i.e., GXGXXG) is characteristic of receptor protein tyrosine kinases.

The extracellular domain may also be identified through commonly recognized criteria of extracellular amino acid sequences. The determination of appropriate criteria is known to those skilled in the art, and has been described, for example, by Hopp et al, Proc. Nat'l Acad. Sci. USA 78, 3824-3828 (1981); Kyte et al, J. Mol. Biol. 157, 105-132 (1982); Emini, J. Virol. 55, 836-839 (1985); Jameson et al, CA BIOS 4, 181-186 (1988); and Karplus et al, Naturwissenschaften 72, 212-213 (1985). Amino acid domains predicted by these criteria to be surface exposed characteristic of extracellular domains.

As will be discussed in more detail below, the nucleic acid molecules that encode the receptors of the invention may be inserted into known vectors for use in standard recombinant DNA techniques. Standard recombinant DNA techniques are those such as are described in Sambrook et al., "Molecular Cloning," Second Edition, Cold Spring Harbor Laboratory Press (1987) and by Ausubel et al., Eds, "Current Protocols in Molecular Biology," Green Publishing Associates and Wiley-Interscience, New York (1987). The vectors may be circular (i.e. plasmids) or non-circular. Standard vectors are available for cloning and expression in a host. The host may be prokaryotic or eucaryotic. Prokaryotic hosts are preferably E. coli. Preferred eucaryotic hosts include yeast, insect and mammalian cells. Preferred mammalian cells include, for example, CHO, COS and human cells.

Ligands

The invention also includes ligands that bind to the receptor pTKs of the invention. In addition to binding, the ligands stimulate the proliferation of additional primitive stem cells, differentiation into more mature progenitor cells, or both.

The ligand may be a growth factor that occurs naturally in a mammal, preferably the same mammal that produces the corresponding receptor. The growth factor may be isolated and purified, or be present on the surface of an isolated population of cells, such as stromal cells. A partial amino acid sequence of a Flk2 ligand is AQSLSFXTKFDLD, wherein X is any amino acid. (See SEQ. ID. NO. 11)

The ligand may also be a molecule that does not occur naturally in a mammal. For example, antibodies, preferably monoclonal, raised against the receptors of the invention or against anti-ligand antibodies mimic the shape of, and act as, ligands if they constitute the negative image of the receptor or anti-ligand antibody binding site. The ligand may also be a non-protein molecule that acts as a ligand when it binds to, or otherwise comes into contact with, the receptor.

In another embodiment, nucleic acid molecules encoding the ligands of the invention are provided. The nucleic acid molecule may be RNA, DNA or cDNA.

Stimulating Proliferation of Stem Cells

The invention also includes a method of stimulating the proliferation and/or differentiation of primitive mammalian hematopoietic stem cells as defined above. The method comprises contacting the stem cells with a ligand in accordance with the

present invention. The stimulation of proliferation and/or differentiation may occur in vitro or in vivo.

5 The ability of a ligand according to the invention to stimulate proliferation of stem cells in vitro and in vivo has important therapeutic applications. Such applications include treating mammals, including humans, whose primitive stem cells do not sufficiently undergo self-renewal. Example of such medical problems include those that occur when defects in hematopoietic stem cells or their related growth factors depress the number of white blood cells. Examples of such medical problems include anemia, such as macrocytic and aplastic anemia. Bone marrow damage resulting from cancer chemotherapy and radiation is another example of a medical problem that would be helped by the stem cell factors of the invention.

Functional Equivalents

20 The invention includes functional equivalents of the pTK receptors, receptor domains, and ligands described above as well as of the nucleic acid sequences encoding them. A protein is considered a functional equivalent of another protein for a specific function if the equivalent protein is immunologically cross-reactive with, and has the same function as, the receptors and ligands of the invention. The equivalent may, for example, be a fragment of the protein, or a substitution, addition or deletion mutant of the protein.

30 For example, it is possible to substitute amino acids in a sequence with equivalent amino acids. Groups of amino acids known normally to be equivalent are:

- 35 (a)Ala(A) Ser(S) Thr(T) Pro(P) Gly(G);
(b)Asn(N) Asp(D) Glu(E) Gln(Q);
(c)His(H) Arg(R) Lys(K);

(d)Met(M) Leu(L) Ile(I) Val(V); and
(e)Phe(F) Tyr(Y) Trp(W).

5 Substitutions, additions and/or deletions in the receptors
and ligands may be made as long as the resulting equivalent
receptors and ligands are immunologically cross reactive with,
and have the same function as, the native receptors and ligands.

10 The equivalent receptors and ligands will normally have
substantially the same amino acid sequence as the native
receptors and ligands. An amino acid sequence that is
substantially the same as another sequence, but that differs from
the other sequence by means of one or more substitutions,
additions and/or deletions is considered to be an equivalent
15 sequence. Preferably, less than 25%, more preferably less than
10%, and most preferably less than 5% of the number of amino acid
residues in the amino acid sequence of the native receptors and
ligands are substituted for, added to, or deleted from.

20 Equivalent nucleic acid molecules include nucleic acid
sequences that encode equivalent receptors and ligands as defined
above. Equivalent nucleic acid molecules also include nucleic
acid sequences that differ from native nucleic acid sequences in
ways that do not affect the corresponding amino acid sequences.

25

ISOLATION OF NUCLEIC ACID MOLECULES AND PROTEINS

Isolation of Nucleic Acid Molecules Encoding Receptors

30 In order to produce nucleic acid molecules encoding
mammalian stem cell receptors, a source of stem cells is
provided. Suitable sources include fetal liver, spleen, or
thymus cells or adult marrow or brain cells.

35 For example, suitable mouse fetal liver cells may be

obtained at day 14 of gestation. Mouse fetal thymus cells may be obtained at day 14-18, preferably day 15, of gestation. Suitable fetal cells of other mammals are obtained at gestation times corresponding to those of mouse.

5

Total RNA is prepared by standard procedures from stem cell receptor-containing tissue. The total RNA is used to direct cDNA synthesis. Standard methods for isolating RNA and synthesizing cDNA are provided in standard manuals of molecular biology such as, for example, in Sambrook et al., "Molecular Cloning," Second Edition, Cold Spring Harbor Laboratory Press (1987) and in Ausubel et al., (Eds), "Current Protocols in Molecular Biology," Greene Associates/Wiley Interscience, New York (1990).

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The cDNA of the receptors is amplified by known methods. For example, the cDNA may be used as a template for amplification by polymerase chain reaction (PCR); see Saiki et al., Science, 239, 487 (1988) or Mullis et al., U.S. patent 4,683,195. The sequences of the oligonucleotide primers for the PCR amplification are derived from the sequences of known receptors, such as from the sequences given in Figures 1a and 1b for Flk2 and in Figure 2 for Flk1, preferably from Flk2. (See SEQ. ID. NOS. 1, 3 and 5, respectively) The oligonucleotides are synthesized by methods known in the art. Suitable methods include those described by Caruthers in Science 230, 281-285 (1985).

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In order to isolate the entire protein-coding regions for the receptors of the invention, the upstream oligonucleotide is complementary to the sequence at the 5' end, preferably encompassing the ATG start codon and at least 5-10 nucleotides upstream of the start codon. The downstream oligonucleotide is complementary to the sequence at the 3' end, optionally encompassing the stop codon. A mixture of upstream and downstream oligonucleotides are used in the PCR amplification.

The conditions are optimized for each particular primer pair according to standard procedures. The PCR product is analyzed by electrophoresis for the correct size cDNA corresponding to the sequence between the primers.

5

Alternatively, the coding region may be amplified in two or more overlapping fragments. The overlapping fragments are designed to include a restriction site permitting the assembly of the intact cDNA from the fragments.

10

The amplified DNA encoding the receptors of the invention may be replicated in a wide variety of cloning vectors in a wide variety of host cells. The host cell may be prokaryotic or eukaryotic. The DNA may be obtained from natural sources and, optionally, modified, or may be synthesized in whole or in part.

15

The vector into which the DNA is spliced may comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences. Some suitable prokaryotic cloning vectors include plasmids from E. coli, such as colE1, pCR1, pBR322, pMB9, pUC, pKSM, and RP4. Prokaryotic vectors also include derivatives of phage DNA such as M13 and other filamentous single-stranded DNA phages.

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25 Isolation of Receptors

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DNA encoding the receptors of the invention are inserted into a suitable vector and expressed in a suitable prokaryotic or eucaryotic host. Vectors for expressing proteins in bacteria, especially E.coli, are known. Such vectors include the PATH vectors described by Dieckmann and Tzagoloff in J. Biol. Chem. 260, 1513-1520 (1985). These vectors contain DNA sequences that encode anthranilate synthetase (TrpE) followed by a polylinker at the carboxy terminus. Other expression vector systems are based on beta-galactosidase (pEX); lambda P_L; maltose binding protein

(pMAL); and glutathione S-transferase (pGST) - see Gene 67, 31 (1988) and Peptide Research 3, 167 (1990).

5 Vectors useful in yeast are available. A suitable example is the 2 μ plasmid.

Suitable vectors for use in mammalian cells are also known. Such vectors include well-known derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences and shuttle vectors derived from
10 combination of functional mammalian vectors, such as those described above, and functional plasmids and phage DNA.

Further eukaryotic expression vectors are known in the art (e.g., P.J. Southern and P. Berg, J. Mol. Appl. Genet. 1, 327-341 (1982); S. Subramani et al, Mol. Cell. Biol. 1, 854-864 (1981);
15 R.J. Kaufmann and P.A. Sharp, "Amplification And Expression Of Sequences Cotransfected with A Modular Dihydrofolate Reductase Complementary DNA Gene," J. Mol. Biol. 159, 601-621 (1982); R.J. Kaufmann and P.A. Sharp, Mol. Cell. Biol. 159, 601-664 (1982);
20 S.I. Scahill et al, "Expression And Characterization Of The Product Of A Human Immune Interferon DNA Gene In Chinese Hamster Ovary Cells," Proc. Natl. Acad. Sci. USA 80, 4654-4659 (1983); G. Urlaub and L.A. Chasin, Proc. Natl. Acad. Sci. USA 77, 4216-4220, (1980).

25 The expression vectors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in
30 order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the glycolytic promoters of yeast,
35 e.g., the promoter for 3-phosphoglycerate kinase, the promoters

of yeast acid phosphatase, e.g., Pho5, the promoters of the yeast
alpha-mating factors, and promoters derived from polyoma,
adenovirus, retrovirus, and simian virus, e.g., the early and
late promoters or SV40, and other sequences known to control the
expression of genes of prokaryotic or eukaryotic cells and their
viruses or combinations thereof.

Vectors containing the receptor-encoding DNA and control
signals are inserted into a host cell for expression of the
receptor. Some useful expression host cells include well-known
prokaryotic and eukaryotic cells. Some suitable prokaryotic
hosts include, for example, E. coli, such as E. coli SG-936, E.
coli HB 101, E. coli W3110, E. coli X1776, E. coli X2282, E. coli
DHI, and E. coli MRC1, Pseudomonas, Bacillus, such as Bacillus
subtilis, and Streptomyces. Suitable eukaryotic cells include
yeast and other fungi, insect, animal cells, such as COS cells
and CHO cells, human cells and plant cells in tissue culture.

The human homologs of the mouse receptors described above
are isolated by a similar strategy. RNA encoding the receptors
are obtained from a source of human cells enriched for primitive
stem cells. Suitable human cells include fetal spleen, thymus
and liver cells, and umbilical cord blood as well as adult brain
and bone marrow cells. The human fetal cells are preferably
obtained on the day of gestation corresponding to mid-gestation
in mice. The amino acid sequences of the human flk receptors as
well as of the nucleic acid sequences encoding them are
homologous to the amino acid and nucleotide sequences of the
mouse receptors.

In the present specification, the sequence of a first
protein, such as a receptor or a ligand, or of a nucleic acid
molecule that encodes the protein, is considered homologous to a
second protein or nucleic acid molecule if the amino acid or
nucleotide sequence of the first protein or nucleic acid molecule

is at least about 30% homologous, preferably at least about 50% homologous, and more preferably at least about 65% homologous to the respective sequences of the second protein or nucleic acid molecule. In the case of proteins having high homology, the amino acid or nucleotide sequence of the first protein or nucleic acid molecule is at least about 75% homologous, preferably at least about 85% homologous, and more preferably at least about 95% homologous to the amino acid or nucleotide sequence of the second protein or nucleic acid molecule.

Combinations of mouse oligonucleotide pairs are used as PCR primers to amplify the human homologs from the cells to account for sequence divergence. The remainder of the procedure for obtaining the human flk homologs are similar to those described above for obtaining mouse flk receptors. The less than perfect homology between the human flk homologs and the mouse oligonucleotides is taken into account in determining the stringency of the hybridization conditions.

Assay for expression of Receptors on Stem Cells

In order to demonstrate the expression of flk receptors on the surface of primitive hematopoietic stem cells, antibodies that recognize the receptor are raised. The receptor may be the entire protein as it exists in nature, or an antigenic fragment of the whole protein. Preferably, the fragment comprises the predicted extra-cellular portion of the molecule.

Antigenic fragments may be identified by methods known in the art. Fragments containing antigenic sequences may be selected on the basis of generally accepted criteria of potential antigenicity and/or exposure. Such criteria include the hydrophilicity and relative antigenic index, as determined by surface exposure analysis of proteins. The determination of appropriate criteria is known to those skilled in the art, and

has been described, for example, by Hopp et al, Proc. Nat'l Acad. Sci. USA 78, 3824-3828 (1981); Kyte et al, J. Mol. Biol. 157, 105-132 (1982); Emini, J. Virol. 55, 836-839 (1985); Jameson et al, CA BIOS 4, 181-186 (1988); and Karplus et al, Naturwissenschaften 72, 212-213 (1985). Amino acid domains predicted by these criteria to be surface exposed are selected preferentially over domains predicted to be more hydrophobic or hidden.

The proteins and fragments of the receptors to be used as antigens may be prepared by methods known in the art. Such methods include isolating or synthesizing DNA encoding the proteins and fragments, and using the DNA to produce recombinant proteins, as described above.

Fragments of proteins and DNA encoding the fragments may be chemically synthesized by methods known in the art from individual amino acids and nucleotides. Suitable methods for synthesizing protein fragments are described by Stuart and Young in "Solid Phase Peptide Synthesis," Second Edition, Pierce Chemical Company (1984). Suitable methods for synthesizing DNA fragments are described by Caruthers in Science 230, 281-285 (1985).

If the receptor fragment defines the epitope, but is too short to be antigenic, it may be conjugated to a carrier molecule in order to produce antibodies. Some suitable carrier molecules include keyhole limpet hemocyanin, Ig sequences, TrpE, and human or bovine serum albumen. Conjugation may be carried out by methods known in the art. One such method is to combine a cysteine residue of the fragment with a cysteine residue on the carrier molecule.

The antibodies are preferably monoclonal. Monoclonal antibodies may be produced by methods known in the art. These

methods include the immunological method described by Kohler and Milstein in Nature 256, 495-497 (1975) and Campbell in "Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas" in Burdon et al., Eds, Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam (1985); as well as by the recombinant DNA method described by Huse et al in Science 246, 1275-1281 (1989).

Polyclonal or monoclonal antisera shown to be reactive with receptor-encoded native proteins, such as with Flk1 and Flk2 encoded proteins, expressed on the surface of viable cells are used to isolate antibody-positive cells. One method for isolating such cells is flow cytometry; see, for example, Loken et al., European patent application 317,156. The cells obtained are assayed for stem cells by engraftment into radiation-ablated hosts by methods known in the art; see, for example, Jordan et al., Cell 61, 953-963 (1990).

Criteria for Novel Stem Cell Receptor Tyrosine Kinases Expressed in Stem Cells

Additional novel receptor tyrosine kinase cDNAs are obtained by amplifying cDNAs from stem cell populations using oligonucleotides as PCR primers; see above. Examples of suitable oligonucleotides are PTK1 and PTK2, which were described by Wilks et al. in Proc. Natl. Acad. Sci. USA 86, 1603-1607 (1989). Novel cDNA is selected on the basis of differential hybridization screening with probes representing known kinases. The cDNA clones hybridizing only at low stringency are selected and sequenced. The presence of the amino acid triplet DFG confirms that the sequence represents a kinase. The diagnostic methionine residue in the WMAPES motif is indicative of a receptor-like kinase, as described above. Potentially novel sequences obtained are compared to available sequences using databases such as

Genbank in order to confirm uniqueness. Gene-specific oligonucleotides are prepared as described above based on the sequence obtained. The oligonucleotides are used to analyze stem cell enriched and depleted populations for expression. Such cell populations in mice are described, for example, by Jordan et al. in Cell 61, 953-956 (1990); Ikuta et al. in Cell 62, 863-864 (1990); Spangrude et al. in Science 241, 58-62 (1988); and Szilvassy et al. in Blood 74, 930-939 (1989). Examples of such human cell populations are described as CD33⁻CD34⁺ by Andrews et al. in the Journal of Experimental Medicine 169, 1721-1731 (1989). Other human stem cell populations are described, for example, in Civin et al., European Patent Application 395,355 and in Loken et al., European Patent Application 317,156.

Isolating Ligands and Nucleic Acid Molecules Encoding Ligands

Cells that may be used for obtaining ligands include stromal cells, for example stromal cells from fetal liver, fetal spleen, fetal thymus and fetal or adult bone marrow. Cell lines expressing ligands are established and screened.

For example, cells such as stromal (non-hematopoietic) cells from fetal liver are immortalized by known methods. Examples of known methods of immortalizing cells include transduction with a temperature sensitive SV40 T-antigen expressed in a retroviral vector. Infection of fetal liver cells with this virus permits the rapid and efficient establishment of multiple independent cell lines. These lines are screened for ligand activity by methods known in the art, such as those outlined below.

Ligands for the receptors of the invention, such as Flk1 and Flk2, may be obtained from the cells in several ways. For example, a bioassay system for ligand activity employs chimeric tagged receptors; see, for example, Flanagan et al., Cell 63,

185-194 (1990). One strategy measures ligand binding directly via a histochemical assay. Fusion proteins comprising the extracellular receptor domains and secretable alkaline phosphatase (SEAP) are constructed and transfected into suitable cells such as NIH/3T3 or COS cells. Flanagan et al. refer to such DNA or amino acid constructs as APTag followed by the name of the receptor - i.e. APTag-c-kit. The fusion proteins bind with high affinity to cells expressing surface-bound ligand. Binding is detectable by the enzymatic activity of the alkaline phosphatase secreted into the medium. The bound cells, which are often stromal cells, are isolated from the APTag-receptor complex.

For example, some stromal cells that bind APTag-Flk1 and APTag-Flk2 fusion proteins include mouse fetal liver cells (see example 1); human fetal spleen cells (see example 3); and human fetal liver (example 3). Some stromal fetal thymus cells contain Flk1 ligand (example 3).

To clone the cDNA that encodes the ligand, a cDNA library is constructed from the isolated stromal cells in a suitable expression vector, preferably a phage such as CDM8, pSV Sport (BRL Gibco) or pIH3, (Seed et al., Proc. Natl. Acad. Sci. USA 84, 3365-3369 (1987)). The library is transfected into suitable host cells, such as COS cells. Cells containing ligands on their surface are detected by known methods, see above.

In one such method, transfected COS cells are distributed into single cell suspensions and incubated with the secreted alkaline phosphatase-flk receptor fusion protein, which is present in the medium from NIH/3T3 or COS cells prepared by the method described by Flanagan et al., see above. Alkaline phosphatase-receptor fusion proteins that are not bound to the cells are removed by centrifugation, and the cells are panned on plates coated with antibodies to alkaline phosphatase. Bound

cells are isolated following several washes with a suitable wash reagent, such as 5% fetal bovine serum in PBS, and the DNA is extracted from the cells. Additional details of the panning method described above may be found in an article by Seed et al.,
5 Proc. Natl. Acad. Sci. USA 84, 3365-3369 (1987).

In a second strategy, the putative extracellular ligand binding domains of the receptors are fused to the transmembrane and kinase domains of the human c-fms tyrosine kinase and
10 introduced into 3T3 fibroblasts. The human c-fms kinase is necessary and sufficient to transduce proliferative signals in these cells after appropriate activation i.e. with the Flk1 or Flk2 ligand. The 3T3 cells expressing the chimeras are used to screen putative sources of ligand in a cell proliferation assay.
15

An alternate approach for isolating ligands using the fusion receptor-expressing 3T3 cells and insertional activation is also possible. A retrovirus is introduced into random chromosomal positions in a large population of these cells. In a small
20 fraction, the retrovirus is inserted in the vicinity of the ligand-encoding gene, thereby activating it. These cells proliferate due to autocrine stimulation of the receptor. The ligand gene is "tagged" by the retrovirus, thus facilitating its isolation.
25

Examples

Example 1. Cells containing mouse Flk1 and Flk2 ligands. Murine stromal cell line 2018.

30

In order to establish stromal cell lines, fetal liver cells are disaggregated with collagen and grown in a mixture of
35 Dulbecco's Modified Eagle's Medium (DMEM) and 10% heat-inactivated fetal calf serum at 37°C. The cells are immortalized

by standard methods. A suitable method involves introducing DNA encoding a growth regulating- or oncogene-encoding sequence into the target host cell. The DNA may be introduced by means of transduction in a recombinant viral particle or transfection in a plasmid. See, for example, Hammerschmidt et al., Nature 340, 393-397 (1989) and Abcouwer et al, Biotechnology 7, 939-946 (1989). Retroviruses are the preferred viral vectors, although SV40 and Epstein-Barr virus can also serve as donors of the growth-enhancing sequences. A suitable retrovirus is the ecotropic retrovirus containing a temperature sensitive SV40 T-antigen (tsA58) and a G418 resistance gene described by McKay in Cell 66, 713-729 (1991). After several days at 37°C, the temperature of the medium is lowered to 32°C. Cells are selected with G418 (0.5 mg/ml). The selected cells are expanded and maintained.

A mouse stromal cell line produced by this procedure is called 2018 and was deposited on October 30, 1991 in the American Type Culture Collection, Rockville, Maryland, USA (ATCC); accession number CRL 10907.

Example 2. Cells containing human Flk1 and Flk2 ligands.

Human fetal liver (18, 20, and 33 weeks after abortion), spleen (18 weeks after abortion), or thymus (20 weeks after abortion) is removed at the time of abortion and stored on ice in a balanced salt solution. After mincing into 1 mm fragments and forcing through a wire mesh, the tissue is washed one time in Hanks Balanced Salt Solution (HBSS).

The disrupted tissue is centrifuged at 200 xg for 15 minutes at room temperature. The resulting pellet is resuspended in 10-20 ml of a tissue culture grade trypsin-EDTA solution (Flow Laboratories). The resuspended tissue is transferred to a

sterile flask and stirred with a stirring bar at room temperature for 10 minutes. One ml of heat-inactivated fetal bovine calf serum (Hyclone) is added to a final concentration of 10% in order to inhibit trypsin activity. Collagenase type IV (Sigma) is added from a stock solution (10 mg/ml in HBSS) to a final concentration of 100 ug/ml in order to disrupt the stromal cells. The tissue is stirred at room temperature for an additional 2.5 hours; collected by centrifugation (400xg, 15 minutes); and resuspended in "stromal medium," which contains Iscove's modification of DMEM supplemented with 10% heat-inactivated fetal calf serum, 5% heat-inactivated human serum (Sigma), 4 mM L-glutamine, 1x sodium pyruvate, (stock of 100x Sigma), 1x non-essential amino acids (stock of 100x, Flow), and a mixture of antibiotics kanomycin, neomycin, penicillin, streptomycin. Prior to resuspending the pellet in the stromal medium, the pellet is washed one time with HBSS. It is convenient to suspend the cells in 60 ml of medium. The number of cultures depends on the amount of tissue.

Example 3. Isolating Stromal cells

Resuspended Cells (example 2) that are incubated at 37°C with 5% carbon dioxide begin to adhere to the plastic plate within 10-48 hours. Confluent monolayers may be observed within 7-10 days, depending upon the number of cells plated in the initial inoculum. Non-adherent and highly refractile cells adhering to the stromal cell layer as colonies are separately removed by pipetting and frozen. Non-adherent cells are likely sources of populations of self-renewing stem cells containing Flk2. The adherent stromal cell layers are frozen in aliquots for future studies or expanded for growth in culture.

An unexpectedly high level of APTag-Flk2 fusion protein binding to the fetal spleen cells is observed. Two fetal spleen lines are grown in "stromal medium," which is described in

example 2.

Non-adherent fetal stem cells attach to the stromal cells and form colonies (colony forming unit - CFU). Stromal cells and CFU are isolated by means of sterile glass cylinders and expanded in culture. A clone, called Fsp 62891, contains the Flk2 ligand. Fsp 62891 was deposited in the American Type Culture Collection, Rockville, Maryland, U.S.A on November 21, 1991, accession number CRL 10935.

Fetal liver and fetal thymus cells are prepared in a similar way. Both of these cell types produce ligands of Flk1 and, in the case of liver, some Flk2. One such fetal thymus cell line, called F.thy 62891, and one such fetal liver cell line, called FL 62891, were deposited in the American Type Culture Collection, Rockville, Maryland, U.S.A on November 21, 1991 and April 2, 1992, respectively, accession numbers CRL 10936 and CRL 11005, respectively.

Stable human cell lines are prepared from fetal cells with the same temperature sensitive immortalizing virus used to prepare the murine cell line described in example 1.

Example 4. Isolation of human stromal cell clone

Highly refractile cells overgrow patches of stromal cells, presumably because the stromal cells produce factors that allow the formation of the CFU. To isolate stromal cell clones, sterile glass cylinders coated with vacuum grease are positioned over the CFU. A trypsin-EDTA solution (100 ml) is added in order to detach the cells. The cells are added to 5 ml of stromal medium and each (clone) plated in a single well of 6-well plate.

Example 5. Plasmid (AP-tag) for expressing secretatable alkaline phosphatase (SEAP)

5 Plasmids that express secretatable alkaline phosphatase are described by Flanagan and Leder in Cell 63, 185-194 (1990). The plasmids contain a promoter, such as the LTR promoter; a polylinker, including HindIII and BglII; DNA encoding SEAP; a poly-A signal; and ampicillin resistance gene; and replication
10 site.

Example 6. Plasmid for expressing APtag-Flk2 and APtag-Flk1 fusion proteins

15 Plasmids that express fusion proteins of SEAP and the extracellular portion of either Flk1 or Flk2 are prepared in accordance with the protocols of Flanagan and Leader in Cell 63, 185-194 (1990) and Berger et al., Gene 66, 1-10 (1988). Briefly,
20 a HindIII-Bam HI fragment containing the extracellular portion of Flk1 or Flk2 is prepared and inserted into the HindIII-BglII site of the plasmid described in example 5.

Example 7. Production Of APtag-Flk1 Or -Flk2 Fusion Protein

25 The plasmids from Example 6 are transfected into Cos-7 cells by DEAE-dextran (as described in Current Protocols in Molecular Biology, Unit 16.13, "Transient Expression of Proteins Using Cos Cells," 1991); and cotransfected with a selectable marker, such
30 as pSV7neo, into NIH/3T3 cells by calcium precipitation. The NIH/3T3 cells are selected with 600µg/ml G418 in 100 mm plates. Over 300 clones are screened for secretion of placental alkaline phosphatase activity. The assay is performed by heating a
35 portion of the supernatant at 65°C for 10 minutes to inactivate background phosphatase activity, and measuring the OD₄₀₅ after incubating with 1M diethanolamine (pH 9.8), 0.5 mM MgCl₂, 10 mM L-homoarginine (a phosphatase inhibitor), 0.5 mg/ml BSA, and 12

5 mM p-nitrophenyl phosphate. Human placental alkaline phosphatase is used to perform a standard curve. The APTaq-Flk1 clones (F-1AP21-4) produce up to 10 µg alkaline phosphatase activity/ml and the APTaq-Flk2 clones (F-2AP26-0) produce up to 0.5 µg alkaline phosphatase activity/ml.

Example 8. Assay For APTaq-Flk1 Or APTaq-Flk2 Binding To Cells

10 The binding of APTaq-Flk1 or APTaq-Flk2 to cells containing the appropriate ligand is assayed by standard methods. See, for example, Flanagan and Leder, Cell 63:185-194, 1990). Cells (i.e., mouse stromal cells, human fetal liver, spleen or thymus, or various control cells) are grown to confluency in six-well plates and washed with HBHA (Hank's balanced salt solution with 15 0.5 mg/ml BSA, 0.02% NaN₃, 20 mM HEPES, pH 7.0). Supernatants from transfected COS or NIH/3T3 cells containing either APTaq-Flk1 fusion protein, APTaq-Flk2 fusion protein, or APTaq without a receptor (as a control) are added to the cell monolayers and incubated for two hours at room temperature on a rotating 20 platform. The concentration of the APTaq-Flk1 fusion protein, APTaq-Flk2 fusion protein, or APTaq without a receptor is 60 ng/ml of alkaline phosphatase as determined by the standard alkaline phosphatase curve (see above). The cells are then rinsed seven times with HBHA and lysed in 350 µl of 1% Triton X- 25 100, 10 mM Tris-HCl (pH 8.0). The lysates are transferred to a microfuge tube, along with a further 150 µl rinse with the same solution. After vortexing vigorously, the samples are centrifuged for five minutes in a microfuge, heated at 65°C for 12 minutes to inactivate cellular phosphatases, and assayed for 30 phosphatase activity as described previously. Results of experiments designed to show the time and dose responses of binding between stromal cells containing the ligands to Flk2 and Flk1 (2018) and APTaq-Flk2, APTaq-Flk1 and APTaq without receptor (as a control) are shown in Figures 3 and 4, respectively.

Example 8A. Plasmids for expressing Flk1/fms and Flk2/fms fusion proteins

5 Plasmids that express fusion proteins of the extracellular portion of either Flk1 or Flk2 and the intracellular portion of c-fms (also known as colony-stimulating factor-1 receptor) are prepared in a manner similar to that described under Example 6 (Plasmid for expressing APTag-Flk2 and APTag-Flk1 fusion
10 proteins). Briefly, a Hind III - Bam HI fragment containing the extracellular portion of Flk1 or Flk2 is prepared and inserted into the Hind III - Bgl II site of a pLH expression vector containing the intracellular portion of c-fms.

15 8B. Expression of Flk1/fms or Flk2/fms in 3T3 cells

The plasmids from Example 8A are transfected into NIH/3T3 cells by calcium. The intracellular portion of c-fms is detected
20 by Western blotting.

Example 9. Cloning and Expression of cDNA Coding For Mouse Ligand To Flk1 and Flk2 Receptors

25 cDNA expressing mouse ligand for Flk1 and Flk2 is prepared by known methods. See, for example, Seed, B., and Aruffo, A. PNAS 84:3365-3369, 1987; Simmons, D. and Seed, B. J. Immunol. 141:2797-2800; and D'Andrea, A.D., Lodish, H.F. and Wong, G.G. Cell 57:277-285, 1989).

The protocols are listed below in sequence: (a) RNA isolation; (b) poly A RNA preparation; (c) cDNA synthesis; (d)
35 cDNA size fractionation; (e) propagation of plasmids (vector); (f) isolation of plasmid DNA; (g) preparation of vector pSV Sport (BRL Gibco) for cloning; (h) compilation of buffers for the above steps; (i) Transfection of cDNA encoding Ligands in Cos 7 Cells;

(j) panning procedure; (k) Expression cloning of Flk1 or Flk2 ligand by establishment of an autocrine loop.

9a. Guanidinium thiocyanate/LiCl Protocol for RNA Isolation

5 For each ml of mix desired, 0.5 g guanidine thiocyanate (GuSCN) is dissolved in 0.55 ml of 25% LiCl (stock filtered through 0.45 micron filter). 20 μ l of mercaptoethanol is added. (The resulting solution is not good for more than about a week at
10 room temperature.)

15 The 2018 stromal cells are centrifuged, and 1 ml of the solution described above is added to up to 5×10^7 cells. The cells are sheared by means of a polytron until the mixture is non-viscous. For small scale preparations ($<10^8$ cells), the sheared mixture is layered on 1.5 ml of 5.7M CsCl (RNase free; 1.26 g CsCl added to every ml 10 mM EDTA pH8), and overlaid with RNase-free water if needed. The mixture is spun in an SW55 rotor at 50 krpm for 2 hours. For large scale preparations, 25 ml of
20 the mixture is layered on 12 ml CsCl in an SW28 tube, overlaid as above, and spun at 24 krpm for 8 hours. The contents of the tube are aspirated carefully with a sterile pasteur pipet connected to a vacuum flask. Once past the CsCl interface, a band around the tube is scratched with the pipet tip to prevent creeping of the layer on the wall down the tube. The remaining CsCl solution is
25 aspirated. The resulting pellet is taken up in water, but not redissolved. 1/10 volume of sodium acetate and three volumes of ethanol are added to the mixture, and spun. The pellet is resuspended in water at 70°C, if necessary. The concentration of the RNA is adjusted to 1 mg/ml and frozen.
30

It should be noted that small RNA molecules (e.g., 5S) do not come down. For small amounts of cells, the volumes are scaled down, and the mixture is overlaid with GuSCN in RNase-free
35 water on a gradient (precipitation is inefficient when RNA is

dilute).

9b. Poly A⁺ RNA preparation

5 (All buffers mentioned are compiled separately below)

A disposable polypropylene column is prepared by washing with 5M NaOH and then rinsing with RNase-free water. For each milligram of total RNA, approximately 0.3 ml (final packed bed) of oligo dT cellulose is added. The oligo dT cellulose is prepared by resuspending approximately 0.5 ml of dry powder in 1 ml of 0.1M NaOH and transferring it into the column, or by percolating 0.1M NaOH through a previously used column. The column is washed with several column volumes of RNase-free water until the pH is neutral, and rinsed with 2-3 ml of loading buffer. The column bed is transferred to a sterile 15 ml tube using 4-6 ml of loading buffer.

Total RNA from the 2018 cell line is heated to 70°C for 2-3 minutes. LiCl from RNase-free stock is added to the mixture to a final concentration of 0.5M. The mixture is combined with oligo dT cellulose in the 15 ml tube, which is vortexed or agitated for 10 minutes. The mixture is poured into the column, and washed with 3 ml loading buffer, and then with 3 ml of middle wash buffer. The mRNA is eluted directly into an SW55 tube with 1.5 ml of 2 mM EDTA and 0.1% SDS, discarding the first two or three drops.

The eluted mRNA is precipitated by adding 1/10 volume of 3M sodium acetate and filling the tube with ethanol. The contents of the tube are mixed, chilled for 30 minutes at -20°C, and spun at 50 krpm at 5°C for 30 minutes. After the ethanol is decanted, and the tube air dried, the mRNA pellet is resuspended in 50-100 µl of RNase-free water. 5 µl of the resuspended mRNA is heated to 70°C in MOPS/EDTA/formaldehyde, and examined on an RNase-free 1% agarose gel.

9c. cDNA Synthesis

The protocol used is a variation of the method described by Gubler and Hoffman in Gene 25, 263-270 (1983).

1. First Strand. 4 μ g of mRNA is added to a microfuge tube, heated to approximately 100°C for 30 seconds, quenched on ice. The volume is adjusted to 70 μ l with RNase-free water. 20 μ l of RT1 buffer, 2 μ l of RNase inhibitor (Boehringer 36 u/ μ l), 1 μ l of 5 μ g/ μ l of oligo dT (Collaborative Research), 2.5 μ l of 20 mM dXTP's (ultrapure - US Biochemicals), 1 μ l of 1M DTT and 4 μ l of RT-XL (Life Sciences, 24 u/ μ l) are added. The mixture is incubated at 42°C for 40 minutes, and inactivated by heating at 70°C for 10 minutes.

2. Second Strand. 320 μ l of RNase-free water, 80 μ l of RT2 buffer, 5 μ l of DNA Polymerase I (Boehringer, 5 U/ μ l), 2 μ l RNase H (BRL 2 u/ μ l) are added to the solution containing the first strand. The solution is incubated at 15°C for one hour and at 22°C for an additional hour. After adding 20 μ l of 0.5M EDTA, pH 8.0, the solution is extracted with phenol and precipitated by adding NaCl to 0.5M linear polyacrylamide (carrier) to 20 μ g/ml, and filling the tube with EtOH. The tube is spun for 2-3 minutes in a microfuge, vortexed to dislodge precipitated material from the wall of the tube, and respun for one minute.

3. Adaptors. Adaptors provide specific restriction sites to facilitate cloning, and are available from BRL Gibco, New England Biolabs, etc. Crude adaptors are resuspended at a concentration of 1 μ g/ μ l. MgSO₄ is added to a final concentration of 10 mM, followed by five volumes of EtOH. The resulting precipitate is rinsed with 70% EtOH and resuspended in TE at a concentration of 1 μ g/ μ l. To kinase, 25 μ l of resuspended adaptors is added to 3 μ l of 10X kinasing buffer and 20 units of kinase. The mixture is incubated at 37°C overnight. The precipitated cDNA is

resuspended in 240 μ l of TE (10/1). After adding 30 μ l of 10X low salt buffer, 30 μ l of 10X ligation buffer with 0.1mM ATP, 3 μ l (2.4 μ g) of kinased 12-mer adaptor sequence, 2 μ l (1.6 μ g) of kinased 8-mer adaptor sequence, and 1 μ l of T4 DNA ligase (BioLabs, 400 u/ μ l, or Boehringer, 1 Weiss unit ml), the mixture is incubated at 15°C overnight. The cDNA is extracted with phenol and precipitated as above, except that the extra carrier is omitted, and resuspended in 100 μ l of TE.

10 9d. cDNA Size Fractionation.

A 20% KOAc, 2 mM EDTA, 1 μ g/ml ethidium bromide solution and a 5% KOAc, 2 mM EDTA, 1 μ g/ml ethidium bromide solution are prepared. 2.6 ml of the 20% KOAc solution is added to the back chamber of a small gradient maker. Air bubbles are removed from the tube connecting the two chambers by allowing the 20% solution to flow into the front chamber and forcing the solution to return to the back chamber by tilting the gradient maker. The passage between the chambers is closed, and 2.5 ml of 5% solution is added to the front chamber. Any liquid in the tubing from a previous run is removed by allowing the 5% solution to flow to the end of the tubing, and then to return to its chamber. The apparatus is placed on a stirplate, and, with rapid stirring, the topcock connecting the two chambers and the front stopcock are opened. A polyallomer 5W55 tube is filled from the bottom with the KOAc solution. The gradient is overlaid with 100 μ l of cDNA solution, and spun for three hours at 50k rpm at 22°C. To collect fractions from the gradient, the SW55 tube is pierced close to the bottom of the tube with a butterfly infusion set (with the luer hub clipped off). Three 0.5 ml fractions and then six 0.25 ml fractions are collected in microfuge tubes (approximately 22 and 11 drops, respectively). The fractions are precipitated by adding linear polyacrylamide to 20 μ g/ml and filling the tube to the top with ethanol. The tubes are cooled, spun in a microfuge tube for three minutes, vortexed, and respun

for one minute. The resulting pellets are rinsed with 70% ethanol and respun, taking care not to permit the pellets to dry to completion. Each 0.25 ml fraction is resuspended in 10 μ l of TE, and 1 μ l is run on a 1% agarose minigel. The first three fractions, and the last six which contain no material smaller than 1 kb are pooled.

9e. Propagation of Plasmids

SupF plasmids are selected in nonsuppressing bacterial hosts containing a second plasmid, p3, which contains amber mutated ampicillin and tetracycline drug resistance elements. See Seed, Nucleic Acids Res., 11, 2427-2445 (1983). The p3 plasmid is derived from RP1, is 57 kb in length, and is a stably maintained, single copy episome. The ampicillin resistance of this plasmid reverts at a high rate so that amp^r plasmids usually cannot be used in p3-containing strains. Selection for tetracycline resistance alone is almost as good as selection for ampicillin-tetracycline resistance. However, spontaneous appearance of chromosomal suppressor tRNA mutations presents an unavoidable background (frequency about 10^{-9}) in this system. Colonies arising from spontaneous suppressor mutations are usually larger than colonies arising from plasmid transformation. Suppressor plasmids are selected in Luria broth (LB) medium containing ampicillin at 12.5 μ g/ml and tetracycline at 7.5 μ g/ml. For scaled-up plasmid preparations, M9 Casamino acids medium containing glycerol (0.8%) is employed as a carbon source. The bacteria are grown to saturation.

Alternatively, pSV Sport (BRL, Gaithersburg, Maryland) may be employed to provide SV40 derived sequences for replication, transcription initiation and termination in COS 7 cells, as well as those sequences necessary for replication and ampicillin resistance in E. coli.

9f. Isolation of Vector DNA/Plasmid

One liter of saturated bacterial cells are spun down in J6 bottles at 4.2k rpm for 25 minutes. The cells are resuspended in 40 ml 10 mM EDTA, pH 8. 80 ml 0.2M NaOH and 1% SDS are added, and the mixture is swirled until it is clear and viscous. 40 ml 5M KOAc, pH 4.7 (2.5M KOAc, 2.5M HOAc) is added, and the mixture is shaken semi-vigorously until the lumps are approximately 2-3 mm in size. The bottle is spun at 4.2k rpm for 5 minutes. The supernatant is poured through cheesecloth into a 250 ml bottle, which is then filled with isopropyl alcohol and centrifuged at 4.2k rpm for 5 minutes. The bottle is gently drained and rinsed with 70% ethanol, taking care not to fragment the pellet. After inverting the bottle and removing traces of ethanol, the mixture is resuspended in 3.5 ml Tris base/EDTA (20 mM/10 mM). 3.75 ml of resuspended pellet and 0.75 ml 10 mg/ml ethidium bromide are added to 4.5 g CsCl. VTi80 tubes are filled with solution, and centrifuged for at least 2.5 hours at 80k rpm. Bands are extracted by visible light with 1 ml syringe and 20 gauge or lower needle. The top of the tube is cut off with scissors, and the needle is inserted upwards into the tube at an angle of about 30 degrees with respect to the tube at a position about 3 mm beneath the band, with the bevel of the needle up. After the band is removed, the contents of the tube are poured into bleach. The extracted band is deposited in a 13 ml Sarstedt tube, which is then filled to the top with n-butanol saturated with 1M NaCl extract. If the amount of DNA is large, the extraction procedure may be repeated. After aspirating the butanol into a trap containing 5M NaOH to destroy ethidium, an approximately equal volume of 1M ammonium acetate and approximately two volumes of 95% ethanol are added to the DNA, which is then spun at 10k rpm for 5 minutes. The pellet is rinsed carefully with 70% ethanol, and dried with a swab or lyophilizer.

9g. Preparation of Vector for Cloning

20 µg of vector is cut in a 200 µl reaction with 100 units of BstXI (New York Biolabs) at 50°C overnight in a well thermostated, circulating water bath. Potassium acetate solutions (5 and 20%) are prepared in 5W55 tubes as described above. 100 µl of the digested vector is added to each tube and spun for three hours, 50k rpm at 22°C. Under 300 nm UV light, the desired band is observed to migrate 2/3 of the length of the tube. Forward trailing of the band indicates that the gradient is overloaded. The band is removed with a 1 ml syringe fitted with a 20 gauge needle. After adding linear polyacrylamide and precipitating the plasmid by adding three volumes of ethanol, the plasmid is resuspended in 50 µl of TE. Trial ligations are carried out with a constant amount of vector and increasing amounts of cDNA. Large scale ligation are carried out on the basis of these trial ligations. Usually the entire cDNA prep requires 1-2 µg of cut vector.

9h. Buffers

Loading Buffer: .5M LiCl, 10 mM Tris pH 7.5, 1 mM EDTA .1% SDS.
Middle Wash Buffer: .15M LiCl, 10 mM Tris pH 7.5, 1 mM EDTA .1% SDS.

RT1 Buffer: .25M Tris pH 8.8 (8.2 at 42°), .25M KCl, 30 mM MgCl₂.
RT2 Buffer: .1M Tris pH 7.5, 25 mM MgCl₂, .5M KCl, .25 mg/ml BSA, 50 mM dithiothreitol (DTT).

10X Low Salt: 60 mM Tris pH 7.5, 60 mM MgCl₂, 50 mM NaCl, 2.5 mg/ml BSA 70 mM DME

10X Ligation Additions: 1 mM ATP, 20 mM DTT, 1 mg/ml BSA 10 mM spermidine.

10X Kinasing Buffer: .5M Tris pH 7.5, 10 mM ATP, 20 mM DTT, 10 mM spermidine, 1 mg/ml BSA 100 mM MgCl₂

9i. Transfection of cDNA encoding Ligands in Cos 7 Cells

Cos 7 cells are split 1:5 into 100 mm plates in Dulbecco's modified Eagles medium (DME)/10% fetal calf serum (FCS), and allowed to grow overnight. 3 ml Tris/DME (0.039M Tris, pH 7.4 in DME) containing 400 µg/ml DEAE-dextran (Sigma, D-9885) is prepared for each 100 mm plate of Cos 7 cells to be transfected. 10 µg of plasmid DNA preparation per plate is added. The medium is removed from the Cos-7 cells and the DNA/DEAE-dextran mixture is added. The cells are incubated for 4.5 hours. The medium is removed from the cells, and replaced with 3 ml of DME containing 2% fetal calf serum (FCS) and 0.1 mM chloroquine. The cells are incubated for one hour. After removing the chloroquine and replacing with 1.5 ml 20% glycerol in PBS, the cells are allowed to stand at room temperature for one minute. 3 ml Tris/DME is added, and the mixture is aspirated and washed two times with Tris/DME. 10 ml DME/10% FCS is added and the mixture is incubated overnight. The transfected Cos 7 cells are split 1:2 into fresh 100 mm plates with (DME)/10% FCS and allowed to grow.

9j. Panning Procedure for Cos 7 cells Expressing Ligand1) Antibody-coated plates:

25

Bacteriological 100 mm plates are coated for 1.5 hours with rabbit anti-human placental alkaline phosphatase (Dako, California) diluted 1:500 in 10 ml of 50 mM Tris.HCl, pH 9.5. The plates are washed three times with 0.15M NaCl, and incubated with 3 mg BSA/ml PBS overnight. The blocking solution is aspirated, and the plates are utilized immediately or frozen for later use.

35

2) Panning cells:

The medium from transfected Cos 7 cells is aspirated, and 3 ml PBS/0.5 mM EDTA/0.02% sodium azide is added. The plates are incubated at 37°C for thirty minutes in order to detach the cells. The cells are trituated vigorously with a pasteur pipet and collected in a 15 ml centrifuge tube. The plate is washed with a further 2 ml PBS/EDTA/azide solution, which is then added to the centrifuge tube. After centrifuging at 200 xg for five minutes, the cells are resuspended in 3 ml of APTaq-Flk1 (F-1AP21-4) or Flk2 (F-2AP26-0) supernatant from transfected NIH/3T3 cells (see Example 7.), and incubated for 1.5 hours on ice. The cells are centrifuged again at 200 xg for five minutes. The supernatant is aspirated, and the cells are resuspended in 3 ml PBS/EDTA/azide solution. The cell suspension is layered carefully on 3 ml PBS/EDTA/azide/2% Ficoll, and centrifuged at 200 xg for four minutes. The supernatant is aspirated, and the cells are resuspended in 0.5 ml PBS/EDTA/azide solution. The cells are added to the antibody-coated plates containing 4 ml PBS/EDTA/azide/5% FBS, and allowed to stand at room temperature one to three hours. Non-adhering cells are removed by washing gently two or three times with 3 ml PBS/5% FBS.

3) Hirt Supernatant:

0.4 ml 0.6% SDS and 10 mM EDTA are added to the panned plates, which are allowed to stand 20 minutes. The viscous mixture is added by means of a pipet into a microfuge tube. 0.1 ml 5M NaCl is added to the tube, mixed, and chilled on ice for at least five hours. The tube is spun for four minutes, and the supernatant is removed carefully. The contents of the tube are extracted with phenol once, or, if the first interface is not clean, twice. Ten micrograms of linear polyacrylamide (or other carrier) is added, and the tube is filled to the top with ethanol. The resulting precipitate is resuspended in 0.1 ml

water or TE. After adding 3 volumes of EtOH/NaOAc, the cells are reprecipitated and resuspended in 0.1 ml water or TE. The cDNA obtained is transfected into any suitable E. coli host by electroporation. Suitable hosts are described in various catalogs, and include MC1061/p3 or Electromax DH10B Cells of BRL Gibco. The cDNA is extracted by conventional methods.

The above panning procedure is repeated until a pure E. coli clone bearing the cDNA as a unique plasmid recombinant capable of transfecting mammalian cells and yielding a positive panning assay is isolated. Normally, three repetitions are sufficient.

9k. Expression cloning of Flk1 or Flk2 ligand by establishment of an autocrine loop

Cells expressing Flk1/fms or Flk2/fms (Example 10) are transfected with 20-30 µg of a cDNA library from either Flk1 ligand or Flk2 ligand expressing stromal cells, respectively. The cDNA library is prepared as described above (a-h). The cells are co-transfected with 1 µg pLTR neo cDNA. Following transfection the cells are passaged 1:2 and cultured in 800 µg/ml of G418 in Dulbecco's medium (DME) supplemented with 10% CS. Approximately 12 days later the colonies of cells are passaged and plated onto dishes coated with poly -D- lysine (1 mg/ml) and human fibronectin (15 µg/ml). The culture medium is defined serum-free medium which is a mixture (3:1) of DME and Ham's F12 medium. The medium supplements are 8 mM NaHCO₃, 15 mM HEPES pH 7.4, 3 mM histidine, 4 µM MnCl₂, 10 uM ethanolamine, 0.1 µM selenous acid, 2 µM hydrocortisone, 5 µg/ml transferrin, 500 µg/ml bovine serum albumin/linoleic acid complex, and 20 µg/ml insulin (Ref. Zhan, X, et al. Oncogene 1: 369-376, 1987). The cultures are refed the next day and every 3 days until the only cells capable of growing under the defined medium condition remain. The remaining colonies of cells are expanded and tested for the presence of the ligand by assaying for binding of APTag -

Flk1 or APTag - Flk2 to the cells (as described in Example 8). The DNA would be rescued from cells demonstrating the presence of the Flk1 or Flk2 ligand and the sequence.

5 **Example 10. Expression of Ligand cDNA**

10 The cDNA is sequenced, and expressed in a suitable host cell, such as a mammalian cell, preferably COS, CHO or NIH/3T3 cells. The presence of the ligand is confirmed by demonstrating binding of the ligand to APTag-Flk2 fusion protein (see above).

15 **Example 11. Chemical Cross Linking of Receptor and Ligand**

20 Cross linking experiments are performed on intact cells using a modification of the procedure described by Blume-Jensen et al et al., EMBO J., 10, 4121-4128 (1991). Cells are cultured in 100mm tissue culture plates to subconfluence and washed once with PBS-0.1% BSA.

25 To examine chemical cross linking of soluble receptor to membrane-bound ligand, stromal cells from the 2018 stromal cell line are incubated with conditioned media (CM) from transfected 3T3 cells expressing the soluble receptor Flk2-APtag. Cross linking studies of soluble ligand to membrane bound receptor are performed by incubating conditioned media from 2018 cells with transfected 3T3 cells expressing a Flk2-fms fusion construct.

30 Binding is carried out for 2 hours either at room temperature with CM containing 0.02% sodium azide to prevent receptor internalization or at 4°C with CM (and buffers) supplemented with sodium vanadate to prevent receptor dephosphorylation. Cells are washed twice with PBS-0.1% BSA and four times with PBS.

35 Cross linking is performed in PBS containing 250 mM

disuccinimidyl suberate (DSS; Pierce) for 30 minutes at room temperature. The reaction is quenched with Tris-HCL pH7.4 to a final concentration of 50 mM.

5 Cells are solubilized in solubilization buffer: 0.5% Triton - X100, 0.5% deoxycholic acid, 20 mM Tris pH 7.4, 150 mM NaCl, 10mM EDTA, 1mM PMFS, 50 mg/ml aprotinin, 2 mg/ml bestatin, 2 mg/ml pepstatin and 10mg/ml leupeptin. Lysed cells are immediately transferred to 1.5 ml Nalgene tubes and solubilized
10 by rolling end to end for 45 minutes at 4°C. Lysates are then centrifuged in a microfuge at 14,000g for 10 minutes. Solubilized cross linked receptor complexes are then retrieved from lysates by incubating supernatants with 10% (v/v) wheat germ lectin-Sepharose 6MB beads (Pharmacia) at 4°C for 2 hours or
15 overnight.

Beads are washed once with Tris-buffered saline (TBS) and resuspended in 2X SDS-polyacrylamide nonreducing sample buffer. Bound complexes are eluted from the beads by heating at 95°C for
20 5 minutes. Samples are analyzed on 4-12% gradient gels (NOVEX) under nonreducing and reducing conditions (0.35 M 2-mercaptoethanol) and then transferred to PVDF membranes for 2 hours using a Novex blotting apparatus. Blots are blocked in TBS-3% BSA for 1 hour at room temperature followed by incubation
25 with appropriate antibody.

Cross linked Flk2-APtag and Flk2-fms receptors are detected using rabbit polyclonal antibodies raised against human alkaline phosphatase and fms protein, respectively. The remainder of the
30 procedure is carried out according to the instructions provided in the ABC Kit (Pierce). The kit is based on the use of a biotinylated secondary antibody and avidin-biotinylated horseradish peroxidase complex for detection.

35

Example 12. Expression and purification of Flag-Flk2.

1. Design of the Flag-Flk2 expression plasmids.

5 A synthetic DNA fragment (Fragment 1) is synthesized using complementary oligonucleotides BP1 and BP2 (see below and SEQ. ID. NOS. 7 and 8). The fragment encoded the following features in the 5' to 3' order: Sal I restriction site, 22 base pair (bp) 5' untranslated region containing an eukaryotic ribosome binding
10 site, an ATG initiation codon, preprotrypsinogen signal sequence, coding region for the FLAG peptide (DYKDDDDKI) and Bgl II restriction site.

15 A cDNA fragment (Fragment 2) encoding Asn 27 to Ser 544 of murine Flk2 is obtained by polymerase chain reaction (PCR) using primers designed to introduce an in frame Bgl II site at the 5' end (oligonucleotide BP5, see below and SEQ. ID. NO. 9) and a termination codon followed by a Not I site at the 3' end (oligonucleotide BP10, see below and SEQ. ID. NO. 10). The
20 template for the PCR reaction is full length Flk2 cDNA (Matthews et al., Cell 65:1143 (1991)). Fragment 2 is extensively digested with Bgl II and Not I restriction enzymes prior to ligation.

25 To assemble the complete Flag-Flk2 gene, Fragments 1 and 2 are ligated in a tripartate ligation into Sal I and Not I digested plasmid pSPORT (Gibco/BRL, Grand Island, NY) to give the plasmid pFlag-Flk2.

30 Preferably, the Flag-Flk2 protein is attached at either end to the Fc portion of an immunoglobulin (Ig). The Ig is preferably attached to the Flk2 portion of the Flag-Flk2 protein. To assemble the construct pFlag-Flk2-Ig, the sequences coding for the CH¹ domain of human immunoglobulin G (IgG¹) are placed
35 downstream of the Flk2 coding region in the plasmid pFlag-Flk2 as per the method described by Zettlemeissl et al., DNA and Cell

Biology 9: 347-352 (1990).

The sequences of oligonucleotides used to construct the Flag-Flk2 gene are given below:

5

Oligonucleotide BP1:

5'-AATTCGTCGACTTTCTGTCACCATGAGTGCACCTTCTGATCCTAGCCCTTGTG
GGAGCTGCTGTTGCTGACTACAAAGATGATGATGACAAGATCTA-3'

10

Oligonucleotide BP2:

5'-AGCTTAGATCTTGTCATCATCATCTTTGTAGTCAGCAACAGCAGCTCCCACA
AGGGCTAGGATCAGAAGTGCACCTCATGGTGACAGAAAGTCGACG-3'

Oligonucleotide BP5:

15

5'-TGAGAAGATCTCAAACCAAGACCTGCCTGT-3'

Oligonucleotide BP10:

5'-CCAATGGCGGCCGCTCAGGAGATGTTGTCTTGGA-3'

20

(See SEQ. ID. NOS. 7-10, respectively)

2. Expression of the Flag-Flk2 construct.

25

For transient expression of the Flag-Flk2 construct, the Sal I to Not I fragment from pFlag-Flk2 is subcloned into the plasmid pSVSPORT (Gibco/BRL) to give the plasmid pSVFlag-Flk2. For expression of the Flag-Flk2 protein pSVFlag-Flk2 is transfected into COS monkey cells using the DEAE-dextran method.

30

For stable expression in eukaryotic cells, the Sal I-Not I fragment of pFlag-Flk2 is cloned into the EcoRV and Not I sites of the plasmid pcDNA I/Neo (Invitrogen Co., San Diego, CA). The Sal I 3' recessed terminus of pFlag-Flk2 is filled with the Klenow fragment of DNA polymerase I and a mixture of

35

deoxyribonucleotides to make the site compatible with the EcoRV site of the vector. The resulting construct is introduced into cultured mammalian cells using either the Lipofectin (Gibco/BRL) or the calcium phosphate methods.

5

For expression in insect cells, the SalI to Hind III (from pSPORT polylinker) fragment of pFlag-Flk2 is subcloned into the BamHI-Hind III sites of the baculovirus transfer vector pBlueBac III (Invitrogen). The vector Bam HI site and the insert Sal I site are blunted with Klenow (see above). Production of the recombinant virus and infection of the Sf9 insect cells is performed as per manufacturers directions (Invitrogen).

10

Expression of the Flag-Flk2 protein is detected by Western blotting of SDS-PAGE separated conditioned media (mammalian cells) or cell lysates (insect cells) with the anti-Flag monoclonal antibody (mAb) M1 (International Biotechnology, Inc. [IBI], New Haven, CT).

15

3. Affinity purification of the Flag-Flk2 protein from conditioned media or insect cell lysates is performed using immobilized mAb M1 (IBI) as per manufacturers specifications.

20

3.1 Affinity purification of the Flag-Flk2-Ig¹ protein from conditioned media is performed using immobilized Protein A (Pharmacia LKB, Piscataway, NJ) as per the manufacturers instructions.

25

II. Use of the Flag-Flk2 protein to search for the Flk2 ligand.

30

1. Binding and cross-linking studies to detect membrane-bound ligand:

A. Binding studies.

35

Murine stromal lines (eg. 2018 cells ATCC CRL 10907 (see below), see example 1, *supra*) considered to be candidates for expression of the Flk2 ligand were deposited at the American Type Culture Collection, ATCC CRL 10907 (see below) and cultured in
5 Dulbecco's modified Eagles medium (DMEM; Gibco/BRL) supplemented with 10% fetal calf serum. The cells are grown to confluency in 10 cm plates and washed once with PBS. Conditioned media containing Flag-Flk2 is incubated with the cells at 4°C for 2 hrs. The cell monolayers are rinsed extensively to remove the
10 non-bound protein, solubilized and centrifuged to remove insoluble cellular material. Glycoproteins in the lysates are partially purified with wheat germ agglutinin-Sepharose (Pharmacia LKB, Piscataway, NJ), boiled in an SDS sample buffer, separated on SDS-PAGE gels and transferred to nitrocellulose
15 membranes. The membranes are probed with the M1 antibody to detect the presence of cell-associated Flag-Flk2 protein.

B. In a cross-linking study, the above protocol is followed except that prior to solubilization the monolayer are treated
20 with the crosslinker disuccinimidyl suberate (DSS; Pierce, Rockford, IL). The presence of a putative ligand is detected by an upward shift in the apparent molecular weight of the Flag-Flk2 band on Western blots.

C. Purified Flag-Flk2 protein labelled with Na125I via the Chloramine T method is used to asses the ability of the soluble extracellular domain of the Flk2 receptor to bind transmembrane form of the Flk2 ligand in cultured stromal lines. The labelled protein is added to monolayers of stromal cells on ice for 2 hr
30 in the presence or absence of excess unlabelled protein. Specific binding is calculated by subtracting counts bound in the presence of excess unlabelled protein from the total counts bound.

2. Use of the Flag-Flk2 protein to search for secreted form of
35 the ligand.

5 A. The Flag-Flk2 protein is used in attempts to identify the Flk2 ligand in conditioned media from stromal cell cultures via modification of the direct N-terminal sequencing method of Pan et al., Bioch. Biophys. Res. Comm. 166:201 (1990). Briefly, the Flag-Flk2 protein N-terminally sequenced by automatic Edman degradation chemistry on an ABI 477A sequencer with on line PTH amino acid analysis. Approximately 15 amino acids are determined. The protein is then immobilized on Nugal PAF silica beads via free NH₄⁺ groups. The immobilized Flag-Flk2 is
10 incubated with conditioned media from putative ligand-producing cells for 30 min at 4°C and washed free off non-bound proteins with phosphate buffered saline adjusted to 2M NaCl. The resulting protein complex is resequenced. For each sequencing cycle, any amino acid not expected at this position in the FLAG-Flk2 protein
15 is considered as possibly originating from a protein complexed to the Flk2 receptor.

20 B. For conventional affinity chromatography, the Flag-Flk2 protein is immobilized on a stable support such as Sepharose. 35S-methionine labelled-conditioned media from stromal cell lines are passed over the affinity matrix and bound material is analyzed by SDS-PAGE gel electrophoresis and autoradiography.

25 3. Use of the Flag-Flk2 protein in expression cloning experiments.

30 A method of expression cloning of integral membrane proteins in COS cells has been described (Aruffo and Seed, Proc. Natl. Acad. Sci. 84:8573 (1987)). A cDNA library is prepared from an appropriate stromal cell line such as 2018 and is transfected into COS cells. Cells transiently expressing the Flk2 ligand are affinity adsorbed onto plastic plates coated with the Flag-Flk2 protein. The cells are lysed, the plasmid DNA is recovered and amplified in a bacterial host. The cycle of transfection into COS
35 cells is repeated until a single cDNA clone encoding the ligand

molecule is isolated.

In a modification of the above technique, pools of transfected COS cells are screened for binding of ¹²⁵I-Flag-Flk2. Positive cells pools are selected and plasmid DNA is recovered and amplified in E. coli. The resulting DNA preparation is used in subsequent rounds of transfection and transient expression until all cells are positive for binding of ¹²⁵I-Flag-Flk2. The cDNA in the final plasmid preparation is then sequenced to determine the sequence of the putative Flk-2 ligand.

Example 13 Isolating the Human Flk2 Ligand from PHA-LCM

13a. Source of the human Flk2 ligand

The Flk2 ligand is isolated from tissue culture medium conditioned by phytohemagglutinin-stimulated human peripheral blood leukocytes (PHA-LCM). The medium is prepared by isolating normal human peripheral blood mononuclear cells (leukocytes) from whole blood by density centrifugation (Ficoll-Hypaque, Pharmacia Biotech, Inc, Piscataway, NJ) and incubating these cells at a concentration of 2×10^6 cells/ml with the lectin phytohemagglutinin (PHA, Gibco Laboratories, Grand Island, NY) in a commercially-prepared, serum-free defined culture medium (AIMV; Gibco Laboratories, Grand Island, NY) for one week. PHA-LCM is harvested by removal of cells and debris by centrifugation.

13b. Isolating the human Flk2 ligand from PHA-LCM

The Flk2 ligand is one of a large number of proteins that are specifically secreted by PHA-activated cells into the medium. Several purification steps using conventional chromatographic techniques are required to isolate the Flk2 ligand. The chromatographic columns used (not listed in specific order) include: Blue Sepharose Fast Flow (Pharmacia Biotech, Inc,

Piscataway, NJ) to remove the medium component albumin, anion exchange (Q-Sepharose Fast Flow, Pharmacia Biotech, Inc, Piscataway, NJ) , cation exchange (S-Sepharose Fast Flow, Pharmacia Biotech, Inc, Piscataway, NJ), gel filtration (Superdex 75, Pharmacia Biotech, Inc, Piscataway, NJ), heparin sepharose (Pharmacia Biotech, Inc, Piscataway, NJ), ConA (Pharmacia Biotech, Inc, Piscataway, NJ), wheat germ agglutinin (Pharmacia Biotech, Inc, Piscataway, NJ), and C4 reverse phase (Vydac, The Separations Group, Hesperia, CA).

Biological assays are used throughout the purification to identify which column fractions contain the Flk2 ligand. The Flk2 ligand specifically stimulates proliferation *in vitro* of cell lines transfected with constructs expressing the full length Flk2 receptor or a chimeric receptor comprising of the the extracellular domain of the Flk2 receptor and the intracellular domain of a different protein tyrosine kinase receptor such as *fms*, the receptor for CSF-1. For example, the Flk2 ligand specifically stimulates proliferation of murine NIH 3T3 fibroblast cell line transfected with constructs expressing the murine or human Flk2 receptor in either full length or chimeric form (see example 8B). The parent untransfected 3T3 cells do not respond to the Flk2 ligand. The format of the Flk2 receptor 3T3 cell assay uses 96 well tissue culture plates (Becton Dickinson, Lincoln Park, NJ), where column fractions or other test samples are serially diluted across the plates in wells containing a mixture of AIMV and Dulbecco's modification of Eagle's medium (DMEM, Gibco Laboratories, Grand Island, NY). Samples are tested for their ability to stimulate proliferation of Flk2 receptor 3T3 cells initially cultured at 3×10^4 cells/well. Survival of Flk2 receptor 3T3 cells is dependent on the presence of the Flk2 ligand. Viable Flk2 receptor 3T3 cells are quantitated after three to five days in culture either visually or spectrophotometrically (Molecular Devices Corporation, Menlo Park, CA) using a tetraformazan salt (XTT,

Diagnostic Chemicals Ltd, Oxford, CT) that when cleaved by actively respiring cells forms diformazan salt which absorbs light at a wavelength (450 nm) that is different from the starting compound (560 nm). Relative (units/ml) and specific (units/mg) activities are defined as the reciprocal dilution at which half-maximal stimulation is detected.

13c. Physical properties of the human Flk2 ligand

The human Flk2 ligand isolated from PHA-LCM is a glycosylated protein and has an apparent molecular weight of 18 kDa, as determined by SDS-PAGE analysis run under reducing (β -mercaptoethanol) and non-reducing conditions. Its N-terminal fourteen amino acid sequence is A Q S L S F X F T K F D L D, wherein X is any amino acid. (See SEQ. ID. NO. 11) Its biological activity is inactivated at 100° C but not 60° C in five minutes and the activity is retained after the Flk2 ligand is subjected to a pH of 2.8 at room temperature for two hours.

The 18 kDa Flk2 ligand may act alone, in combination with other cytokines (e.g., interleukin 1, interleukin 3, interleukin 6, interleukin 11 or the kit ligand), or as a component of a complex of proteins that stimulate the Flk2 receptor in transfected 3T3 cell or in primitive hematopoietic progenitors. The complex of proteins may include a soluble or membrane-bound form of the Flk2 receptor.

A radiolabeled form of the Flk2 ligand may be used to detect and to measure the levels of Flk2 receptor, such as the soluble form of the Flk2 receptor, for example, in serum or urine of patients with bone marrow disorders.

13d. Biological activity of the human Flk2 ligand

In addition to acting on Flk2 receptor-expressing 3T3 cells,

the Flk2 ligand specifically stimulates proliferation of cells that naturally express the Flk2 receptor. In assays using either a human myeloid cell line or a subset of primitive hematopoietic progenitors expressing the surface phenotype CD34, the Flk2 ligand promotes proliferation but not differentiation into mature progeny. These observations suggest that the Flk2 ligand alone or in combination with other cytokines (e.g. Interleukin 1, Interleukin 3, Interleukin 6, Interleukin 11, or the kit ligand) may act to preserve or expand primitive hematopoietic progenitors *in vitro* and *in vivo*.

SUPPLEMENTAL ENABLEMENT

The invention as claimed is enabled in accordance with the above specification and readily available references and starting materials. Nevertheless, Applicants have deposited with the American Type Culture Collection, Rockville, Md., USA (ATCC) the cell lines listed below:

2018, ATCC accession no. CRL 10907, deposited October 30, 1991.

Fsp 62891, ATCC accession no. CRL 10935, deposited November 21, 1991.

F.thy 62891, ATCC accession no. CRL 10936, deposited November 21, 1991.

FL 62891, ATCC accession no. CRL 11005, deposited April 2, 1992.

These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and the regulations thereunder (Budapest Treaty). This assures

- 5 maintenance of a viable culture for 30 years from date of deposit. The organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Applicants and ATCC which assures unrestricted availability upon issuance of the pertinent U.S. patent. Availability of the deposited strains is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Lemischka, Ihor R.
- (ii) TITLE OF INVENTION: TOTIPOTENT HEMATOPOIETIC STEM CELL RECEPTORS AND THEIR LIGANDS
- (iii) NUMBER OF SEQUENCES: 11
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: ImClone Systems Incorporated
(B) STREET: 180 Varick Street
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: U.S.A.
(F) ZIP: 10014
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
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(B) FILING DATE: 15-JAN-1993

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/045,272

(B) FILING DATE: 01-APR-1993

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/076022

(B) FILING DATE: 09-JUN-1993

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/080244

(B) FILING DATE: 18-JUN-1993

(vii) PRIOR APPLICATION DATA:
 (A) APPLICATION NUMBER: US 08/081508
 (B) FILING DATE: 21-JUN-1993

(vii) PRIOR APPLICATION DATA:
 (A) APPLICATION NUMBER: US 08/096759
 (B) FILING DATE: 22-JUL-1993

(vii) PRIOR APPLICATION DATA:
 (A) APPLICATION NUMBER: US 08/125669
 (B) FILING DATE: 23-SEP-1993

(viii) ATTORNEY/AGENT INFORMATION:
 (A) NAME: Feit, Irving N.
 (B) REGISTRATION NUMBER: 28,601
 (C) REFERENCE/DOCKET NUMBER: LEM-3-15P

(ix) TELECOMMUNICATION INFORMATION:
 (A) TELEPHONE: 212-645-1405
 (B) TELEFAX: 212-645-2054

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3453 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
(B) LOCATION: 112..3006

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
(B) LOCATION: 31..111

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 31..3009

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCGGCCTGGC TACCGCGCGC TCCGGAGGCC ATG CGG GCG TTG GCG CAG CGC AGC 54
 Met Arg Ala Leu Ala Gln Arg Ser
 -27 -25 -20
 GAC CGG CGG CTG CTG CTT GTT GTT TCA GTA ATG ATT CTT GAG 102
 Asp Arg Arg Leu Leu Leu Val Val Leu Ser Val Met Ile Leu Glu
 -15 -10 -5
 ACC GTT ACA AAC CAA GAC CTG CCT GTG ATC AAG TGT GTT TTA ATC AGT 150
 Thr Val Thr Asn Gln Asp Leu Pro Val Ile Lys Cys Val Leu Ile Ser
 1 5 10
 CAT GAG AAC AAT GGC TCA TCA GCG GGA AAG CCA TCA TCG TAC CGA ATG 198
 His Glu Asn Asn Gly Ser Ser Ala Gly Lys Pro Ser Ser Tyr Arg Met
 15 20 25
 GTG CGA GGA TCC CCA GAA GAC CTC CAG TGT ACC CCG AGG CGC CAG AGT 246
 Val Arg Gly Ser Pro Glu Asp Leu Gln Cys Thr Pro Arg Arg Gln Ser
 30 35 40 45
 GAA GGG ACG GTA TAT GAA GCG GCC ACC GTG GAG GTG GCC GAG TCT GGG 294

Glu Gly Thr Val Tyr Glu Ala Ala Thr Val Glu Val Ala Glu Ser Gly
 50 55 60
 TCC ATC ACC CTG CAA GTG CAG CTC GCC ACC CCA GGG GAC CTT TCC TGC
 Ser Ile Thr Leu Gln Val Gln Leu Ala Thr Pro Gly Asp Leu Ser Cys
 65 70 75 342
 CTC TGG GTC TTT AAG CAC AGC TCC CTG GGC TGC CAG CCG CAC TTT GAT
 Leu Trp Val Phe Lys His Ser Ser Leu Gly Cys Gln Pro His Phe Asp
 80 85 90 390
 TTA CAA AAC AGA GGA ATC GTT TCC ATG GCC ATC TTG AAC GTG ACA GAG
 Leu Gln Asn Arg Gly Ile Val Ser Met Ala Ile Leu Asn Val Thr Glu
 95 100 105 438
 ACC CAG GCA GGA GAA TAC CTA CTC CAT ATT CAG AGC GAA CGC GCC AAC
 Thr Gln Ala Gly Glu Tyr Leu Leu His Ile Gln Ser Glu Arg Ala Asn
 110 115 120 125 486
 TAC ACA GTA CTG TTC ACA GTG AAT GTA AGA GAT ACA CAG CTG TAT GTG
 Tyr Thr Val Leu Phe Thr Val Asn Val Arg Asp Thr Gln Leu Tyr Val
 130 135 140 534
 CTA AGG AGA CCT TAC TTT AGG AAG ATG GAA AAC CAG GAT GCA CTG CTC
 Leu Arg Arg Pro Tyr Phe Arg Lys Met Glu Asn Gln Asp Ala Leu Leu
 145 150 155 582
 TGC ATC TCC GAG GGT GTT CCG GAG CCC ACT GTG GAG TGG GTG CTC TGC
 Cys Ile Ser Glu Gly Val Pro Glu Pro Thr Val Glu Trp Val Leu Cys
 160 165 170 630
 AGC TCC CAC AGG GAA AGC TGT AAA GAA GAA GGC CCT GCT GTT GTC AGA
 Ser Ser His Arg Glu Ser Cys Lys Glu Glu Gly Pro Ala Val Val Arg
 175 180 185 678
 AAG GAG GAA AAG GTA CTT CAT GAG TTG TTC GGA ACA GAC ATC AGA TGC
 Lys Glu Glu Lys Val Leu His Glu Leu Phe Gly Thr Asp Ile Arg Cys
 726

190 195 200 205 774
 TGT GCT AGA AAT GCA CTG GGC CGC GAA TGC ACC AAG CTG TTC ACC ATA
 Cys Ala Arg Asn Ala Leu Gly Arg Glu Cys Thr Lys Leu Phe Thr Ile
 210 215 220
 822
 GAT CTA AAC CAG GCT CCT CAG AGC ACA CTG CCC CAG TTA TTC CTG AAA
 Asp Leu Asn Gln Ala Pro Gln Ser Thr Leu Pro Gln Leu Phe Leu Lys
 225 230 235
 870
 GTG GGG GAA CCC TTG TGG ATC AGG TGT AAG GCC ATC CAT GTG AAC CAT
 Val Gly Glu Pro Leu Trp Ile Arg Cys Lys Ala Ile His Val Asn His
 240 245 250
 918
 GGA TTC GGG CTC ACC TGG GAG CTG GAA GAC AAA GCC CTG GAG GAG GGC
 Gly Phe Gly Leu Thr Trp Trp Glu Leu Glu Asp Lys Ala Leu Glu Glu Gly
 255 260 265
 966
 AGC TAC TTT GAG ATG AGT ACC TAC TCC ACA AAC AAG ACC ATG ATT CGG
 Ser Tyr Phe Glu Met Ser Thr Tyr Ser Thr Asn Arg Thr Met Ile Arg
 270 275 280 285
 1014
 ATT CTC TTG GCC TTT GTG TCT TCC GTG GGA AGG AAC GAC ACC GGA TAT
 Ile Leu Leu Ala Phe Val Ser Ser Val Gly Arg Asn Asp Thr Gly Tyr
 290 295 300
 1062
 TAC ACC TGC TCT TCC TCA AAG CAC CAC CCC AGC CAG TCA GCG TTG GTG ACC
 Tyr Thr Cys Ser Ser Ser Lys His Pro Ser Gln Ser Ala Leu Val Thr
 305 310 315
 1110
 ATC CTA GAA AAA GGG TTT ATA AAC GCT ACC AGC TCG CAA GAA GAG TAT
 Ile Leu Glu Lys Gly Phe Ile Asn Ala Thr Ser Ser Gln Glu Glu Tyr
 320 325 330
 1158
 GAA ATT GAC CCG TAC GAA AAG TTC TGC TTC TCA GTC AGG TTT AAA GCG
 Glu Ile Asp Pro Tyr Glu Lys Phe Cys Phe Ser Val Arg Phe Lys Ala
 335 340 345

TAC CCA CGA ATC CGA TGC ACG TGG ATC TTC TCT CAA GCC TCA TTT CCT 1206
 Tyr Pro Arg Ile Arg Cys Thr 355
 350
 TGT GAA CAG AGA GGC CTG GAG GAT GGG TAC AGC ATA TCT AAA TTT TGC 1254
 Cys Glu Gln Arg Gly Leu Glu Asp Gly Tyr Ser Ile Ser Lys Phe Cys
 370 375 380
 GAT CAT AAG AAC AAG CCA GGA GAG TAC ATA TTC TAT GCA GAA AAT GAT 1302
 Asp His Lys Asn Lys Pro Gly Glu Tyr Ile Phe Tyr Ala Glu Asn Asp
 385 390 395
 GAC GCC CAG TTC ACC AAA ATG TTC ACG CTG AAT ATA AGA AAG AAA CCT 1350
 Asp Ala Gln Phe Thr Lys Met Phe Thr Leu Asn Ile Arg Lys Lys Pro
 400 405 410
 CAA GTG CTA GCA AAT GCC TCA GCC AGC CAG GCG TCC TGT TCC TCT GAT 1398
 Gln Val Leu Ala Asn Ala Ser Ala Ser Gln Ala Ser Cys Ser Ser Asp
 415 420 425
 GGC TAC CCG CTA CCC TCT TGG ACC TGG AAG AAG TGT TCG GAC AAA TCT 1446
 Gly Tyr Pro Leu Pro Ser Trp Thr Trp Lys Lys Cys Ser Asp Lys Ser
 430 435 440 445
 CCC AAT TGC ACG GAG GAA ATC CCA GAA GGA GTT TGG AAT AAA AAG GCT 1494
 Pro Asn Cys Thr Glu Glu Ile Pro Glu Gly Val Trp Asn Lys Lys Ala
 450 455 460
 AAC AGA AAA GTG TTT GGC CAG TGG GTG TCG AGC AGT ACT CTA AAT ATG 1542
 Asn Arg Lys Val Phe Gly Gln Trp Val Ser Ser Thr Leu Asn Met
 465 470 475
 AGT GAG GCC GGG AAA GGG CTT CTG GTC AAA TGC TGT GCG TAC AAT TCT 1590
 Ser Glu Ala Gly Lys Gly Leu Leu Val Lys Cys Cys Ala Tyr Asn Ser
 480 485 490
 ATG GGC ACG TCT TGC GAA ACC ATC TTT TTA AAC TCA CCA GGC CCC TTC 1638

Met Gly Thr Ser Cys Glu Thr Ile Phe Leu Asn Ser Pro Gly Pro Phe
495 500 505

CCT TTC ATC CAA GAC AAC ATC TCC TTC TAT GCG ACC ATT GGG CTC TGT 1686
Pro Phe Ile Gln Asp Asn Ile Ser Phe Tyr Ala Thr Ile Gly Leu Cys
510 515 520 525

CTC CCC TTC ATT GTT GTC CTC ATT GTG TTG ATC TGC CAC AAA TAC AAA 1734
Leu Pro Phe Ile Val Val Leu Ile Val Leu Ile Cys His Lys Tyr Lys
530 535 540 545

AAG CAA TTT AGG TAC GAG AGT CAG CTG CAG ATG ATC CAG GTG ACT GGC 1782
Lys Gln Phe Arg Tyr Glu Ser Gln Leu Gln Met Ile Gln Val Thr Gly
545 550 555

CCC CTG GAT AAC GAG TAC TTC TAC TTT GAC TTC AGG GAC TAT GAA TAT 1830
Pro Leu Asp Asn Glu Tyr Phe Tyr Val Asp Phe Arg Asp Tyr Glu Tyr
560 565 570 575

GAC CTT AAG TGG GAG TTC CCG AGA GAG AAC TTA GAG TTT GGG AAG GTC 1878
Asp Leu Lys Trp Glu Phe Pro Arg Glu Asn Leu Glu Phe Gly Lys Val
580 585

CTG GGG TCT GGC GCT TTC GGG AGG GTG ATG AAC GCC ACC GGC TAT GGC 1926
Leu Gly Ser Gly Ala Phe Gly Arg Val Met Asn Ala Thr Ala Tyr Gly
590 595 600 605

ATT AGT AAA ACG GGA GTC TCA ATT CAG GTG GCG GTG AAG ATG CTA AAA 1974
Ile Ser Lys Thr Gly Val Ser Ile Gln Val Ala Val Lys Met Leu Lys
610 615 620

GAG AAA GCT GAC AGC TGT GAA AAA GAA GCT CTC ATG TCG GAG CTC AAA 2022
Glu Lys Ala Asp Ser Cys Glu Lys Glu Ala Leu Met Ser Glu Leu Lys
625 630 635

ATG ATG ACC CAC CTG GGA CAC CAT GAC AAC ATC GTG AAT CTG CTG GGG 2070
Met Met Thr His Leu Gly His His Asp Asn Ile Val Asn Leu Leu Gly
640 645 650 655

640	645	650	2118
GCA TGC ACA CTG TCA GGG CCA GTG TAC TTG ATT TTT GAA TAT TGT TGC			
Ala Cys Thr Leu Ser Gly Pro Val Tyr Leu Ile Phe Glu Tyr Cys Cys	655	665	
TAT GGT GAC CTC AAC TAC CTA AGA AGT AAA AGA GAG AAG TTT CAC			2166
Tyr Gly Asp Leu Leu Asn Tyr Leu Arg Ser Lys Arg Glu Lys Phe His	670	680	
675			
AGG ACA TGG ACA GAG ATT TTT AAG GAA CAT AAT TTC AGT TCT TAC CCT			2214
Arg Thr Trp Thr Glu Ile Phe Lys Glu His Asn Phe Ser Ser Tyr Pro	690	695	
705			
ACT TTC CAG GCA CAT TCA AAT TCC AGC ATG CCT GGT TCA CGA GAA GTT			2262
Thr Phe Gln Ala His Ser Asn Ser Ser Met Pro Gly Ser Arg Glu Val	710	715	
CAG TTA CAC CCG CCC TTG GAT CAG CTC TCA GGG TTC AAT GGG AAT TCA			2310
Gln Leu His Pro Pro Leu Asp Gln Leu Ser Gly Phe Asn Gly Asn Ser	720	725	
735			
ATT CAT TCT GAA GAT GAG ATT GAA TAT GAA AAC CAG AAG AGG CTG GCA			2358
Ile His Ser Glu Asp Glu Ile Glu Tyr Glu Asn Gln Lys Arg Leu Ala	740	745	
750			
GAA GAA GAG GAG GAA GAT TTG AAC GTG CTG ACG TTT GAA GAC CTC CTT			2406
Glu Glu Glu Glu Asp Leu Asn Val Leu Thr Phe Glu Asp Leu Leu	755	760	
765			
TGC TTT GCG TAC CAA GTG GCC AAA GGC ATG GAA TTC CTG GAG TTC AAG			2454
Cys Phe Ala Tyr Gln Val Ala Lys Gly Met Glu Phe Leu Glu Phe Lys	770	775	
780			
TCG TGT GTC CAC AGA GAC CTG GCA GCC AGG AAT GTG TTG GTC ACC CAC			2502
Ser Cys Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Thr His	785	790	
795			

2550 GGG AAG GTG GTG AAG ATC TGT GAC TTT GGA CTG GCC CGA GAC ATC CTG
 Gly Lys Val Val Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp Ile Leu
 800 805 810
 2598 AGC GAC TCC AGC TAC GTC GTC AGG GGC AAC GCA CGG CTG CCG GTG AAG
 Ser Asp Ser Ser Tyr Val Val Arg Gly Asn Ala Arg Leu Pro Val Lys
 815 820 825
 2646 TGG ATG GCA CCC GAG AGC TTA TTT GAA GGG ATC TAC ACA ATC AAG AGT
 Trp Met Ala Pro Glu Ser Leu Phe Glu Gly Ile Tyr Thr Ile Lys Ser
 830 835 840 845
 2694 GAC GTC TGG TCC TAC GGC ATC CTT CTC TGG GAG ATA TTT TCA CTG GGT
 Asp Val Trp Ser Tyr Gly Ile Leu Leu Trp Glu Ile Phe Ser Leu Gly
 850 855 860
 2742 GTG AAC CCT TAC CCT GGC ATT CCT GTC GAC GCT AAC TTC TAT AAA CTG
 Val Asn Pro Tyr Pro Gly Ile Pro Val Asp Ala Asn Phe Tyr Lys Leu
 865 870 875
 2790 ATT CAG AGT GGA TTT AAA ATG GAG CAG CCA TTC TAT GCC ACA GAA GGG
 Ile Gln Ser Gly Phe Lys Met Glu Gln Pro Phe Tyr Ala Thr Glu Gly
 880 885 890
 2838 ATA TAC TTT GTA ATG CAA TCC TGC TGG GCT TTT GAC TCA AGG AAG CGG
 Ile Tyr Phe Val Met Gln Ser Cys Trp Ala Phe Asp Ser Arg Lys Arg
 895 900 905
 2886 CCA TCC TTC CCC AAC CTG ACT TCA TTT TTA GGA TGT CAG CTG GCA GAG
 Pro Ser Phe Pro Asn Leu Thr Ser Phe Leu Gly Cys Gln Leu Ala Glu
 910 915 920 925
 2934 GCA GAA GAA GCA TGT ATC AGA ACA TCC ATC CAT CTA CCA AAA CAG GCG
 Ala Glu Glu Ala Cys Ile Arg Thr Ser Ile His Leu Pro Lys Gln Ala
 930 935 940
 2982 GCC CCT CAG CAG AGA GGC GGG CTC AGA GCC CAG TCG CCA CAG CGC CAG

Ala Pro Gln Gln Arg Gly Gly Leu Arg Ala Gln Ser Pro Gln Arg Gln
945 950 955

GTG AAG ATT CAC AGA GAA AGA AGT TAGCGAGGAG GCCTTGGACC CCGCCACCCT
Val Lys Ile His Arg Glu Arg Ser 3036
960 965

AGCAGGCTGT AGACCGCAGA GCCAAGATTA GCCTCGCCTC TGAGGAAGCG CCCTACAGCG 3096

CGTTGCTTCG CTGGACTTTT CTCTAGATGC TGTCTGCCAT TACTCCAAAG TGA CTCTCTAT 3156

AAAATCAAAC CTCTCCTCGC ACAGGCGGGA GAGCCAATAA TGAGACTTGT TGGTGAGCCC 3216

GCCTACCCCTG GGGGCCCTTC CACGAGCTTG AGGGGAAAGC CATGTATCTG AAATATAGTA 3276

TATTCTTGTA AATACGTGAA ACAAAACCAA CCCGTTTTTT GCTAAGGGAA AGCTAAATAT 3336

GATTTTAAA AATCTATGTT TTAAAATACT ATGTAAC TTT CATCTATT TAGTGATATA 3396

TTTTATGGAT GGAAATAAAC TTTCTACTGT AAAAAAAAAA AAAAAAAAAA 3453

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 992 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Arg Ala Leu Ala Gln Arg Ser Asp Arg Arg Leu Leu Leu Leu Val
-27 -25 -20 -15

Val Leu Ser Val Met Ile Leu Glu Thr Val Thr Asn Gln Asp Leu Pro

-10	-5	1	5
Val Ile Lys Cys Val Leu Ile Ser His Glu Asn Asn Gly Ser Ser Ala			
	10	15	20
Gly Lys Pro Ser Ser Tyr Arg Met Val Arg Gly Ser Pro Glu Asp Leu			
	25	30	35
Gln Cys Thr Pro Arg Arg Gln Ser Glu Gly Thr Val Tyr Glu Ala Ala			
	40	45	50
Thr Val Glu Val Ala Glu Ser Gly Ser Ile Thr Leu Gln Val Gln Leu			
	55	60	65
Ala Thr Pro Gly Asp Leu Ser Cys Leu Trp Val Phe Lys His Ser Ser			
	70	75	80
Leu Gly Cys Gln Pro His Phe Asp Leu Gln Asn Arg Gly Ile Val Ser			
	90	95	100
Met Ala Ile Leu Asn Val Thr Glu Thr Gln Ala Gly Glu Tyr Leu Leu			
	105	110	115
His Ile Gln Ser Glu Arg Ala Asn Tyr Thr Val Leu Phe Thr Val Asn			
	120	125	130
Val Arg Asp Thr Gln Leu Tyr Val Leu Arg Arg Pro Tyr Phe Arg Lys			
	135	140	145
Met Glu Asn Gln Asp Ala Leu Leu Cys Ile Ser Glu Gly Val Pro Glu			
	150	155	160
Pro Thr Val Glu Trp Val Leu Cys Ser Ser His Arg Glu Ser Cys Lys			
	170	175	180
Glu Glu Gly Pro Ala Val Val Arg Lys Glu Glu Lys Val Leu His Glu			
	185	190	195

Leu Phe Gly Thr Asp Ile Arg Cys Cys Ala Arg Asn Ala Leu Gly Arg
 200 205 210
 Glu Cys Thr Lys Leu Phe Thr Ile Asp Leu Asn Gln Ala Pro Gln Ser
 215 220 225
 Thr Leu Pro Gln Leu Phe Leu Lys Val Gly Glu Pro Leu Trp Ile Arg
 230 235 240 245
 Cys Lys Ala Ile His Val Asn His Gly Phe Gly Leu Thr Trp Glu Leu
 250 255 260
 Glu Asp Lys Ala Leu Glu Glu Gly Ser Tyr Phe Glu Met Ser Thr Tyr
 265 270 275
 Ser Thr Asn Arg Thr Met Ile Arg Ile Leu Leu Ala Phe Val Ser Ser
 280 285 290
 Val Gly Arg Asn Asp Thr Gly Tyr Tyr Thr Cys Ser Ser Ser Lys His
 295 300 305
 Pro Ser Gln Ser Ala Leu val Thr Ile Leu Glu Lys Gly Phe Ile Asn
 310 315 320 325
 Ala Thr Ser Ser Gln Glu Glu Tyr Glu Ile Asp Pro Tyr Glu Lys Phe
 330 335 340
 Cys Phe Ser val Arg Phe Lys Ala Tyr Pro Arg Ile Arg Cys Thr Trp
 345 350 355
 Ile Phe Ser Gln Ala Ser Phe Pro Cys Glu Gln Arg Gly Leu Glu Asp
 360 365 370
 Gly Tyr Ser Ile Ser Lys Phe Cys Asp His Lys Asn Lys Pro Gly Glu
 375 380 385
 Tyr Ile Phe Tyr Ala Glu Asn Asp Asp Ala Gln Phe Thr Lys Met Phe

390	395	400	405
Thr Leu Asn Ile Arg Lys Lys Pro Gln Val Leu Ala Asn Ala Ser Ala			
410	415	420	
Ser Gln Ala Ser Cys Ser Ser Asp Gly Tyr Pro Leu Pro Ser Trp Thr			
425	430	435	
Trp Lys Lys Cys Ser Asp Lys Ser Pro Asn Cys Thr Glu Glu Ile Pro			
440	445	450	
Glu Gly Val Trp Asn Lys Lys Ala Asn Arg Lys Val Phe Gly Gln Trp			
455	460	465	
Val Ser Ser Ser Thr Leu Asn Met Ser Glu Ala Gly Lys Gly Leu Leu			
470	475	480	485
Val Lys Cys Cys Ala Tyr Asn Ser Met Gly Thr Ser Cys Glu Thr Ile			
490	495	500	
Phe Leu Asn Ser Pro Gly Pro Phe Pro Phe Ile Gln Asp Asn Ile Ser			
505	510	515	
Phe Tyr Ala Thr Ile Gly Leu Cys Leu Pro Phe Ile Val Val Leu Ile			
520	525	530	
Val Leu Ile Cys His Lys Tyr Lys Lys Gln Phe Arg Tyr Glu Ser Gln			
535	540	545	
Leu Gln Met Ile Gln Val Thr Gly Pro Leu Asp Asn Glu Tyr Phe Tyr			
550	555	560	565
Val Asp Phe Arg Asp Tyr Glu Tyr Asp Leu Lys Trp Glu Phe Pro Arg			
570	575	580	
Glu Asn Leu Glu Phe Gly Lys Val Leu Gly Ser Gly Ala Phe Gly Arg			
585	590	595	

Val Met Asn Ala Thr Ala Tyr Gly Ile Ser Lys Thr Gly Val Ser Ile
 600 605 610
 Gln Val Ala Val Lys Met Leu Lys Glu Lys Ala Asp Ser Cys Glu Lys
 615 620 625
 Glu Ala Leu Met Ser Glu Leu Lys Met Met Thr His Leu Gly His His
 630 635 640 645
 Asp Asn Ile Val Asn Leu Leu Gly Ala Cys Thr Leu Ser Gly Pro Val
 650 655 660
 Tyr Leu Ile Phe Glu Tyr Cys Cys Tyr Gly Asp Leu Leu Asn Tyr Leu
 665 670 675
 Arg Ser Lys Arg Glu Lys Phe His Arg Thr Trp Thr Glu Ile Phe Lys
 680 685 690
 Glu His Asn Phe Ser Ser Tyr Pro Thr Phe Gln Ala His Ser Asn Ser
 695 700 705
 Ser Met Pro Gly Ser Arg Glu Val Gln Leu His Pro Pro Leu Asp Gln
 710 715 720 725
 Leu Ser Gly Phe Asn Gly Asn Ser Ile His Ser Glu Asp Glu Ile Glu
 730 735 740
 Tyr Glu Asn Gln Lys Arg Leu Ala Glu Glu Glu Glu Asp Leu Asn
 745 750 755
 Val Leu Thr Phe Glu Asp Leu Leu Cys Phe Ala Tyr Gln Val Ala Lys
 760 765 770
 Gly Met Glu Phe Leu Glu Phe Lys Ser Cys Val His Arg Asp Leu Ala
 775 780 785
 Ala Arg Asn Val Leu Val Thr His Gly Lys Val Val Lys Ile Cys Asp

790		795	800	805
Phe Gly Leu Ala Arg Asp Ile Leu Ser Asp Ser Ser Tyr Val Val Arg	810		815	820
Gly Asn Ala Arg Leu Pro Val Lys Trp Met Ala Pro Glu Ser Leu Phe	825		830	835
Glu Gly Ile Tyr Thr Ile Lys Ser Asp Val Trp Ser Tyr Gly Ile Leu	840	845	850	
Leu Trp Glu Ile Phe Ser Leu Gly Val Asn Pro Tyr Pro Gly Ile Pro	855	860	865	
Val Asp Ala Asn Phe Tyr Lys Leu Ile Gln Ser Gly Phe Lys Met Glu	870	875	880	885
Gln Pro Phe Tyr Ala Thr Glu Gly Ile Tyr Phe Val Met Gln Ser Cys		890	895	900
Trp Ala Phe Asp Ser Arg Lys Arg Pro Ser Phe Pro Asn Leu Thr Ser		905	910	915
Phe Leu Gly Cys Gln Leu Ala Glu Ala Glu Glu Ala Cys Ile Arg Thr	920	925	930	
Ser Ile His Leu Pro Lys Gln Ala Ala Pro Gln Gln Arg Gly Gly Leu	935	940	945	
Arg Ala Gln Ser Pro Gln Arg Gln Val Lys Ile His Arg Glu Arg Ser	950	955	960	965

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3501 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 58..3039

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 139..3036

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 58..138

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGAGGGGGCA TCCGAGGGCT GGGCCGGCGC CCTGGGGGAC CCCGGGCTCC GGAGGCC	57
ATG CCG GCG TTG GCG CGC GAC GCG GGC ACC GTG CCG CTG CTC GTT GTT	105
Met Pro Ala Leu Ala Arg Asp Ala Gly Thr Val Pro Leu Leu Val Val	
-27 -25 -20 -15	
TTT TCT GCA ATG ATA TTT GGG ACT ATT ACA AAT CAA GAT CTG CCT GTG	153

Phe Ser Ala Met Ile Phe Gly Thr Ile Thr Asn Gln Asp Leu Pro Val 5
 -10 -5 1
 ATC AAG TGT GTT TTA ATC AAT CAT AAG AAC AAT GAT TCA TCA GTG GGG 201
 Ile Lys Cys Val Leu Ile Asn His Lys Asn Asp Ser Ser Val Gly 20
 10 15
 AAG TCA TCA TCA TAT CCC ATG GTA TCA GAA TCC CCG GAA GAC CTC GGG 249
 Lys Ser Ser Tyr Pro Met Val Ser Glu Ser Pro Glu Asp Leu Gly 35
 25 30
 TGT GCG TTG AGA CCC CAG AGC TCA GGG ACA GTG TAC GAA GCT GCC GCT 297
 Cys Ala Leu Arg Pro Gln Ser Ser Gly Thr Val Tyr Glu Ala Ala 50
 40 45
 GTG GAA GTG GAT GTA TCT GCT TCC ATC ACA CTG CAA GTG CTG GTG GAT 345
 Val Glu Val Asp Val Ser Ala Ser Ile Thr Leu Gln Val Leu Val Asp 65
 55 60
 GCC CCA GGG AAC ATT TCC TGT CTC TGG GTC TTT AAG CAC AGC TCC CTG 393
 Ala Pro Gly Asn Ile Ser Cys Leu Trp Val Phe Lys His Ser Ser Leu 85
 70 75 80
 AAT TGC CAG CCA CAT TTT GAT TTA CAA AAC AGA GGA GTT GTT TCC ATG 441
 Asn Cys Gln Pro His Phe Asp Leu Gln Asn Arg Gly Val Val Ser Met 95
 90
 GTC ATT TTG AAA ATG ACA GAA ACC CAA GCT GGA GAA TAC CTA CTT TTT 489
 Val Ile Leu Lys Met Thr Glu Thr Gln Ala Gly Glu Tyr Leu Leu Phe 110
 105 115
 ATT CAG AGT GAA GCT ACC AAT TAC ACA ATA TTG TTT ACA GTG AGT ATA 537
 Ile Gln Ser Glu Ala Thr Asn Tyr Thr Ile Leu Phe Thr Val Ser Ile 120
 125 130
 AGA AAT ACC CTG CTT TAC ACA TTA AGA AGA CCT TAC TTT AGA AAA ATG 585
 Arg Asn Thr Leu Leu Tyr Thr Leu Arg Arg Pro Tyr Phe Arg Lys Met

135	140	145
GAA AAC CAG GAC GCC CTG GTC TGC ATA TCT GAG AGC GTT CCA GAG CCG Glu Asn Gln Asp Ala Leu Val Cys Ile Ser Glu Ser Val Pro Glu Pro 150 155 160 165	633	
ATC GTG GAA TGG GTG CTT TGC GAT TCA CAG GGG GAA AGC TGT AAA GAA Ile Val Glu Trp Val Leu Cys Asp Ser Gln Gly Glu Ser Cys Lys Glu 170 175 180	681	
GAA AGT CCA GCT GTT AAA AAG GAG GAA AAA GTG CTT CAT GAA TTA Glu Ser Pro Ala Val Lys Lys Glu Glu Lys Val Leu His Glu Leu 185 190 195	729	
TTT GGG ACG GAC ATA AGG TGC TGT GCC AGA AAT GAA CTG GGC AGG GAA Phe Gly Thr Arg Ile Arg Cys Cys Ala Arg Asn Glu Leu Gly Arg Glu 200 205 210	777	
TGC ACC AGG CTG TTC ACA ATA GAT CTA AAT CAA ACT CCT CAG ACC ACA Cys Thr Arg Arg Leu Phe Thr Ile Asp Leu Asn Gln Thr Pro Gln Thr Thr 215 220 225	825	
TTG CCA CAA TTA TTT CTT AAA GTA GGG GAA CCC TTA TGG ATA AGG TGC Leu Pro Gln Leu Phe Leu Lys Val Gly Glu Pro Leu Trp Ile Arg Cys 230 235 240 245	873	
AAA GCT GTT CAT GTG AAC CAT GGA TTC GGG CTC ACC TGG GAA TTA GAA Lys Ala Val His Val Asn His Gly Phe Gly Leu Thr Trp Glu Leu Glu 250 255 260	921	
AAC AAA GCA CTC GAG GAG GGC AAC AAC TTT GAG ATG AGT ACC TAT TCA Asn Lys Ala Ala Leu Glu Glu Gly Asn Tyr Phe Phe Glu Met Ser Thr Tyr Ser 265 270 275	969	
ACA AAC AGA ACT ATG ATA CGG ATT CTG TTT GCT TTT GTA TCA TCA GTG Thr Asn Arg Arg Thr Met Ile Arg Ile Leu Phe Ala Phe val Ser Ser Val 280 285 290	1017	

GCA AGA AAC GAC ACC GGA TAC TAC TAC TGT TCC TCT TCA AAG CAT CCC
 Ala Arg Asn Asp Thr Gly 300 Tyr Tyr Thr Cys Ser Ser Ser Lys His Pro
 295
 1065
 AGT CAA TCA GCT TTG GTT ACC ATC GTA GGA AAG GGA TTT ATA AAT GCT
 Ser Gln Ser Ala Leu Val 315 Thr Ile Val Gly Lys Gly Phe Ile Asn Ala
 310
 1113
 ACC AAT TCA AGT GAA GAT TAT GAA ATT GAC CAA TAT GAA GAG TTT TGT
 Thr Asn Ser Ser Glu Asp Tyr Glu Ile Asp Gln Tyr Glu Glu Phe Cys
 330
 1161
 TTT TCT GTC AGG TTT AAA GCC TAC CCA CAA ATC AGA TGT ACG TGG ACC
 Phe Ser Val Arg Phe Lys Ala Tyr Pro Gln Ile Arg Cys Thr Trp Thr
 345
 1209
 TTC TCT CGA AAA TCA TTT CCT TGT GAG CAA AAG GGT CTT GAT AAC GGA
 Phe Ser Arg Lys Ser Phe Pro Cys Glu Gln Lys Gly Leu Asp Asn Gly
 360
 1257
 TAC AGC ATA TCC AAG TTT TGC AAT CAT AAG CAC CAG CCA GGA GAA TAT
 Tyr Ser Ile Ser Lys Phe Cys Asn His Lys Lys His Gln Pro Gly Glu Tyr
 375
 1305
 ATA TTC CAT GCA GAA AAT GAT GAT GCC CAA TTT ACC AAA ATG TTC ACG
 Ile Phe His Ala Glu Asn Asp Ala Gln Phe Thr Lys Met Phe Thr
 390
 1353
 CTG AAT ATA AGA AGG AAA CCT CAA GTG CTC GCA GAA GCA TCG GCA AGT
 Leu Asn Ile Arg Arg Lys Pro Gln Val Leu Ala Glu Ala Ser Ala Ser
 410
 1401
 CAG GCG TCC TGT TTC TCG GAT GGA TAC CCA TTA CCA TCT TGG ACC TGG
 Gln Ala Ser Cys Phe Ser Asp Gly Tyr Pro Leu Pro Ser Trp Thr Trp
 425
 1449
 AAG AAG TGT TCA GAC AAG TCT CCC AAC TGC ACA GAA GAG ATC ACA GAA
 1497

Lys Lys Cys Ser Asp Lys Ser Pro Asn Cys Thr Glu Glu Ile Thr Glu
 440 445
 GGA GTC TGG AAT AGA AAG GCT AAC AGA AAA GTG TTT GGA CAG TGG GTG
 Gly Val Trp Asn Arg Lys Ala Asn Arg Lys Val Phe Gly Gln Trp Val
 455 460 465 1545
 TCG AGC AGT ACT CTA AAC ATG AGT GAA GCC ATA AAA GGG TTC CTG GTC
 Ser Ser Ser Thr Leu Asn Met Ser Glu Ala Ile Lys Gly Phe Leu Val
 470 475 480 1593
 AAG TGC TGT GCA TAC AAT TCC CTT GGC ACA TCT TGT GAG ACG ATC CTT
 Lys Cys Cys Ala Tyr Asn Ser Leu Gly Thr Ser Cys Glu Thr Ile Leu
 490 495 500 1641
 TTA AAC TCT CCA GGC CCC TTC CCT TTC ATC CAA GAC AAC ATC TCA TTC
 Leu Asn Ser Pro Gly Pro Phe Pro Phe Ile Gln Asp Asn Ile Ser Phe
 505 510 515 1689
 TAT GCA ACA ATT GGT GTT TGT CTC CTC TTC ATT GTC GTT TTA ACC CTG
 Tyr Ala Thr Ile Gly Val Cys Leu Leu Phe Ile Val Val Leu Thr Leu
 520 525 530 1737
 CTA ATT TGT CAC AAG TAC AAA AAG CAA TTT AGG TAT GAA AGC CAG CTA
 Leu Ile Cys His Lys Tyr Lys Lys Gln Phe Arg Tyr Glu Ser Gln Leu
 535 540 545 1785
 CAG ATG GTA CAG GTG ACC GGC TCC TCA GAT AAT GAG TAC TTC TAC GTT
 Gln Met Val Gln Val Thr Gly Ser Ser Asp Asn Glu Tyr Phe Tyr Val
 550 555 560 565 1833
 GAT TTC AGA GAA TAT GAA TAT GAT CTC AAA TGG GAG TTT CCA AGA GAA
 Asp Phe Arg Glu Tyr Glu Tyr Asp Leu Lys Trp Glu Phe Pro Arg Glu
 570 575 580 1881
 AAT TTA GAG TTT GGG AAG GTA CTA GGA TCA GGT GCT TTT GGA AAA GTG
 Asn Leu Glu Phe Gly Lys Val Leu Gly Ser Gly Ala Phe Gly Lys Val
 1929

585	590	595	
ATG AAC GCA ACA GCT TAT GGA ATT AGC AAA ACA GGA GTC TCA ATC CAG Met Asn Ala Thr Ala Tyr Gly Ile Ser Lys Thr Gly Val Ser Ile Gln 600 605 610			1977
GTT GCC GTC AAA ATG CTG AAA GAA AAA GCA GAC AGC TCT GAA AGA GAG Val Ala Val Lys Met Leu Lys Glu Lys Ala Asp Ser Ser Glu Arg Glu 615 620 625			2025
GCA CTC ATG TCA GAA CTC AAG ATG ATG ACC CAG CTG GGA AGC CAC GAG Ala Leu Met Ser Glu Leu Lys Met Met Thr Gln Leu Gly Ser His Glu 630 635 640 645			2073
AAT ATT GTG AAC CTG CTG GGG GCG TGC ACA CTG TCA GGA CCA ATT TAC Asn Ile Val Asn Leu Leu Gly Ala Cys Thr Leu Ser Gly Pro Ile Tyr 650 655 660			2121
TTG ATT TTT GAA TAC TGT TGC TAT GGT GAT CTT CTC AAC TAT CTA AGA Leu Ile Phe Glu Tyr Cys Cys Tyr Gly Asp Leu Leu Asn Tyr Leu Arg 665 670 675			2169
AGT AAA AGA GAA AAA TTT CAC AGG ACT TGG ACA GAG ATT TTC AAG GAA Ser Lys Arg Glu Lys Phe His Arg Thr Trp Thr Glu Ile Phe Lys Glu 680 685 690			2217
CAC AAT TTC AGT TTT TAC CCC ACT TTC CAA TCA CAT CCA AAT TCC AGC His Asn Phe Ser Phe Tyr Pro Thr Phe Gln Ser His Pro Asn Ser Ser 695 700 705			2265
ATG CCT GGT TCA AGA GAA GTT CAG ATA CAC CCG GAC TCG GAT CAA ATC Met Pro Gly Ser Arg Glu Glu Val Gln Ile His Pro Asp Ser Asp Gln Ile 710 715 720 725			2313
TCA GGG CTT CAT GGG AAT TCA TTT CAC TCT GAA GAT GAA ATT GAA TAT Ser Gly Leu His Gly Asn Ser Phe His Ser Glu Asp Glu Ile Glu Tyr 730 735 740			2361

GAA AAC CAA AAA AGG CTG GAA GAA GAG GAG GAG GAC TTG AAT GTG CTT ACA
 Glu Asn Gln Lys Arg Leu Glu Glu Glu Asp Leu Asn Val Leu Thr
 745 750 755 2409

 TTT GAA GAT CTT CTT TGC TTT GCA TAT CAA GTT GCC AAA GGA ATG GAA
 Phe Glu Asp Leu Cys Phe Ala Tyr Gln Val Ala Lys Gly Met Glu
 760 765 770 2457

 TTT CTG GAA TTT AAG TCG TGT GGT CAC AGA GAC CTG GCC GCC AGG AAC
 Phe Leu Glu Phe Lys Ser Cys Val His Arg Asp Leu Ala Ala Arg Asn
 775 780 785 2505

 GTG CTT GTC ACC CAC GGG AAA GTG GTG AAG ATA TGT GAC TTT GGA TTG
 Val Leu Val Thr His Gly Lys Val Val Lys Ile Cys Asp Phe Gly Leu
 790 795 800 2553

 GCT CGA GAT ATC ATG AGT GAT TCC AAC TAT GTT GTC AGG GGC AAT GCC
 Ala Arg Asp Ile Met Ser Asp Ser Asn Tyr Val Val Arg Gly Asn Ala
 810 815 820 2601

 CGT CTG CCT GTA AAA TGG ATG GCC CCC GAA AGC CTG TTT GAA GGC ATC
 Arg Leu Pro Val Lys Trp Met Ala Pro Glu Ser Leu Phe Glu Gly Ile
 825 830 835 2649

 TAC ACC ATT AAG AGT GAT GTC TGG TCA TAT GGA ATA TTA CTG TGG GAA
 Tyr Thr Ile Lys Ser Asp Val Trp Ser Tyr Gly Ile Leu Leu Trp Glu
 840 845 850 2697

 ATC TTC TCA CTT GGT GTG AAT CCT TAC CCT GGC ATT CCG GTT GAT GCT
 Ile Phe Ser Leu Gly Val Asn Pro Tyr Pro Gly Ile Pro Val Asp Ala
 855 860 865 2745

 AAC TTC TAC AAA CTG ATT CAA AAT GGA TTT AAA ATG GAT CAG CCA TTT
 Asn Phe Tyr Lys Leu Ile Gln Asn Gly Phe Lys Met Asp Gln Pro Phe
 870 875 880 2793

 TAT GCT ACA GAA GAA ATA TAC ATT ATA ATG CAA TCC TGC TGG GCT TTT
 2841

Tyr Ala Thr Glu Glu Ile Tyr Ile Ile Met Gln Ser Cys Trp Ala Phe
 890 895 900
 GAC TCA AGG AAA CGG CCA TCC TTC CCT AAT TTG ACT TCG TTT TTA GGA 2889
 Asp Ser Arg Lys Arg Pro Ser Phe Pro Asn Leu Thr Ser Phe Leu Gly
 905 910 915
 TGT CAG CTG GCA GAT GCA GAA GAA GCG ATG TAT CAG AAT GTG GAT GGC 2937
 Cys Gln Leu Ala Asp Ala Glu Glu Ala Met Tyr Gln Asn Val Asp Gly
 920 925 930
 CGT GTT TCG GAA TGT CCT CAC ACC TAC CAA AAC AGG CGA CCT TTC AGC 2985
 Arg Val Ser Glu Cys Pro His Thr Tyr Gln Asn Arg Arg Pro Phe Ser
 935 940 945
 AGA GAG ATG GAT TTG GGG CTA CTC TCT CCG CAG GCT CAG GTC GAA GAT 3033
 Arg Glu Met Asp Leu Gly Leu Ser Pro Gln Ala Gln Val Glu Asp
 950 955 960 965
 TCG TAGAGGAACA ATTTAGTTTTT AAGGACTTCA TCCCTCCACC TATCCCTAAC 3086
 Ser
 AGGCTGTAGA TTACCAAAAC AAGATTAAATT TCATCACTAA AAGAAAATCT ATTATCAACT 3146
 GCTGCTTCAC CAGACTTTTC TCTAGAAGCC GTCTGCGTTT ACTCTTGTTT TCAAAGGGAC 3206
 TTTTGTAAAA TCAAATCATC CTGTCACAAG GCAGGAGGAG CTGATAATGA ACTTTATTGG 3266
 AGCATTGATC TGCATCCAAG GCCTTCTCAG GCCGGCTTGA GTGAATTGTG TACCTGAAGT 3326
 ACAGTATATT CTGTAAATA CATAAAACAA AAGCATTTTG CTAAGGAGAA GCTAATATGA 3386
 TTTTTTAAGT CTATGTTTTA AAATAATATG TAAATTTTTC AGCTATTAG TGATATATTT 3446
 TATGGGTGGG AATAAAATTT CTAACACAGA AAAAAAAAAA AAAAAAAAAA AAAA 3501

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 993 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Pro Ala Leu Ala Arg Asp Ala Gly Thr Val Pro Leu Leu Val Val
-27 -25 -20 -15
Phe Ser Ala Met Ile Phe Gly Thr Ile Thr Asn Gln Asp Leu Pro Val
-10 -5 1 5
Ile Lys Cys Val Leu Ile Asn His Lys Asn Asn Asp Ser Ser Val Gly
10 15 20
Lys Ser Ser Tyr Pro Met Val Ser Glu Ser Pro Glu Asp Leu Gly
25 30 35
Cys Ala Leu Arg Pro Gln Ser Ser Gly Thr Val Tyr Glu Ala Ala Ala
40 45 50
Val Glu Val Asp Val Ser Ala Ser Ile Thr Leu Gln Val Leu Val Asp
55 60 65
Ala Pro Gly Asn Ile Ser Cys Leu Trp Val Phe Lys His Ser Ser Leu
70 75 80 85
Asn Cys Gln Pro His Phe Asp Leu Gln Asn Arg Gly Val Val Ser Met
90 95 100
Val Ile Leu Lys Met Thr Glu Thr Gln Ala Gly Glu Tyr Leu Leu Phe
105 110 115

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Ile Gln Ser Glu Ala Thr Asn Tyr Thr Ile Leu Phe Thr Val Ser Ile
 120 125 130
 Arg Asn Thr Leu Leu Tyr Thr Leu Arg Arg Pro Tyr Phe Arg Lys Met
 135 140 145
 Glu Asn Gln Asp Ala Leu Val Cys Ile Ser Glu Ser Val Pro Glu Pro
 150 155 160 165
 Ile Val Glu Trp Val Leu Cys Asp Ser Gln Gly Glu Ser Cys Lys Glu
 170 175 180
 Glu Ser Pro Ala Val Val Lys Lys Glu Glu Lys Val Leu His Glu Leu
 185 190 195
 Phe Gly Thr Asp Ile Arg Cys Cys Ala Arg Asn Glu Leu Gly Arg Glu
 200 205 210
 Cys Thr Arg Leu Phe Thr Ile Asp Leu Asn Gln Thr Pro Gln Thr Thr
 215 220 225
 Leu Pro Gln Leu Phe Leu Lys Val Gly Glu Pro Leu Trp Ile Arg Cys
 230 235 240 245
 Lys Ala Val His Val Asn His Gly Phe Gly Leu Thr Trp Glu Leu Glu
 250 255 260
 Asn Lys Ala Leu Glu Glu Gly Asn Tyr Phe Glu Met Ser Thr Tyr Ser
 265 270 275
 Thr Asn Arg Thr Met Ile Arg Ile Leu Phe Ala Phe Val Ser Ser Val
 280 285 290
 Ala Arg Asn Asp Thr Gly Tyr Tyr Thr Cys Ser Ser Ser Lys His Pro
 295 300 305
 Ser Gln Ser Ala Leu Val Thr Ile Val Gly Lys Gly Phe Ile Asn Ala

310	315	320	325
Thr Asn Ser Ser Glu Asp Tyr Glu Ile Asp Gln Tyr Glu Glu Phe Cys			
330	335		340
Phe Ser Val Arg Phe Lys Ala Tyr Pro Gln Ile Arg Cys Thr Trp Thr			
345	350		355
Phe Ser Arg Lys Ser Phe Pro Cys Glu Gln Lys Gly Leu Asp Asn Gly			
360	365	370	
Tyr Ser Ile Ser Lys Phe Cys Asn His Lys His Gln Pro Gly Glu Tyr			
375	380	385	
Ile Phe His Ala Glu Asn Asp Ala Gln Phe Thr Lys Met Phe Thr			
390	395	400	405
Leu Asn Ile Arg Arg Lys Pro Gln Val Leu Ala Glu Ala Ser Ala Ser			
	410	415	420
Gln Ala Ser Cys Phe Ser Asp Gly Tyr Pro Leu Pro Ser Trp Thr Trp			
425	430		435
Lys Lys Cys Ser Asp Lys Ser Pro Asn Cys Thr Glu Glu Ile Thr Glu			
440	445	450	
Gly Val Trp Asn Arg Lys Ala Asn Arg Lys Val Phe Gly Gln Trp Val			
455	460	465	
Ser Ser Ser Thr Leu Asn Met Ser Glu Ala Ile Lys Gly Phe Leu Val			
470	475	480	485
Lys Cys Cys Ala Tyr Asn Ser Leu Gly Thr Ser Cys Glu Thr Ile Leu			
	490	495	500
Leu Asn Ser Pro Gly Pro Phe Pro Phe Ile Gln Asp Asn Ile Ser Phe			
	505	510	515

Tyr Ala Thr Ile Gly Val Cys Leu Leu Phe Ile Val Val Leu Thr Leu
 520 525 530
 Leu Ile Cys His Lys Tyr Lys Lys Gln Phe Arg Tyr Glu Ser Gln Leu
 535 540 545
 Gln Met Val Gln Val Thr Gly Ser Ser Asp Asn Glu Tyr Phe Tyr Val
 550 555 560 565
 Asp Phe Arg Glu Tyr Glu Tyr Asp Leu Lys Trp Glu Phe Pro Arg Glu
 570 575 580
 Asn Leu Glu Phe Gly Lys Val Leu Gly Ser Gly Ala Phe Gly Lys Val
 585 590 595
 Met Asn Ala Thr Ala Tyr Gly Ile Ser Lys Thr Gly Val Ser Ile Gln
 600 605 610
 Val Ala Val Lys Met Leu Lys Glu Lys Ala Asp Ser Ser Glu Arg Glu
 615 620 625
 Ala Leu Met Ser Glu Leu Lys Met Met Thr Gln Leu Gly Ser His Glu
 630 635 640 645
 Asn Ile Val Asn Leu Leu Gly Ala Cys Thr Leu Ser Gly Pro Ile Tyr
 650 655 660
 Leu Ile Phe Glu Tyr Cys Cys Tyr Gly Asp Leu Leu Asn Tyr Leu Arg
 665 670 675
 Ser Lys Arg Glu Lys Phe His Arg Thr Trp Thr Glu Ile Phe Lys Glu
 680 685 690
 His Asn Phe Ser Phe Tyr Pro Thr Phe Gln Ser His Pro Asn Ser Ser
 695 700 705
 Met Pro Gly Ser Arg Glu Val Gln Ile His Pro Asp Ser Asp Gln Ile

710	715	720	725
Ser Gly Leu His Gly Asn Ser Phe His Ser Glu Asp Glu Ile Glu Tyr	730	735	740
Glu Asn Gln Lys Arg Leu Glu Glu Glu Asp Leu Asn Val Leu Thr	745	750	755
Phe Glu Asp Leu Leu Cys Phe Ala Tyr Gln Val Ala Lys Gly Met Glu	760	765	770
Phe Leu Glu Phe Lys Ser Cys Val His Arg Asp Leu Ala Ala Arg Asn	775	780	785
Val Leu Val Thr His Gly Lys Val val Lys Ile Cys Asp Phe Gly Leu	790	795	800
Ala Arg Asp Ile Met Ser Ser Asp Ser Asn Tyr Val Val Arg Gly Asn Ala	810	815	820
Arg Leu Pro Val Lys Trp Met Ala Pro Glu Ser Leu Phe Glu Gly Ile	825	830	835
Tyr Thr Ile Lys Ser Asp Val Trp Ser Tyr Gly Ile Leu Leu Trp Glu	840	845	850
Ile Phe Ser Leu Gly Val Asn Pro Tyr Pro Gly Ile Pro Val Asp Ala	855	860	865
Asn Phe Tyr Lys Leu Ile Gln Asn Gly Phe Lys Met Asp Gln Pro Phe	870	875	880
Tyr Ala Thr Glu Glu Ile Tyr Ile Ile Met Gln Ser Cys Trp Ala Phe	890	895	900
Asp Ser Arg Lys Arg Pro Ser Phe Pro Asn Leu Thr Ser Phe Leu Gly	905	910	915

Cys Gln Leu Ala Asp Ala Glu Glu Ala Met Tyr Gln Asn Val Asp Gly
 920 925 930
 Arg Val Ser Glu Cys Pro His Thr Tyr Gln Asn Arg Arg Pro Phe Ser
 935 940 945
 Arg Glu Met Asp Leu Gly Leu Leu Ser Pro Gln Ala Gln Val Glu Asp
 950 955 960 965

Ser

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5406 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 208..4311

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 265..4308

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide

(B) LOCATION: 208..264

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTGTGTCCCG CAGCCGGATA ACCTGGCTGA CCCGATTCCG CGGACACCCG TGCAGCCGCG	60
GCTGGAGCCA GGGCGCCGGT GCCCGGCTC TCCCCGGTCT TGGGCTGCGG GGGCCGATAC	120
CGCCTCTGTG ACTTCTTTGC GGGCCAGGGA CGGAGAAAGGA GTCTGTGCCT GAGAAACTGG	180
GCTCTGTGCC CAGGCGCGAG GTGCAGG ATG GAG AGC AAG GGC CTG CTA GCT	231
Met Glu Ser Lys Gly Leu Leu Ala	
-19 -15	
GTC GCT CTG TGG TTC TGC GTG GAG ACC CGA GCC TCT GTG GGT TTG	279
Val Ala Leu Trp Phe Cys Val Glu Thr Arg Ala Ala Ser Val Gly Leu	
-10 -5 1 5	
CCT GGC GAT TTT CTC CAT CCC CCC AAG CTC AGC ACA CAG AAA GAC ATA	327
Pro Gly Asp Phe Leu His Pro Pro Lys Leu Ser Thr Gln Lys Asp Ile	
10 15 20	
CTG ACA ATT TTG GCA AAT ACA ACC CTT CAG ATT ACT TGC AGG GGA CAG	375
Leu Thr Ile Leu Ala Asn Thr Thr Leu Gln Ile Thr Cys Arg Gly Gln	
25 30 35	
CGG GAC CTG GAC TGG CTT TGG CCC AAT GCT CAG CGT GAT TCT GAG GAA	423
Arg Asp Leu Asp Trp Leu Trp Pro Asn Ala Gln Arg Asp Ser Glu Glu	
40 45 50	
AGG GTA TTG GTG ACT GAA TGC GGC GGT GGT GAC AGT ATC TTC TGC AAA	471
Arg Val Leu Val Thr Glu Cys Gly Gly Asp Ser Ile Phe Cys Lys	
55 60 65	
ACA CTC ACC ATT CCC AGG GTG GTT GGA AAT GAT ACT GGA GCC TAC AAG	519
Thr Leu Thr Ile Pro Arg Val Val Gly Asn Asp Thr Gly Ala Tyr Lys	

70	75	80	85
TGC TCG TAC CGG GAC GTC GAC ATA GCC TCC ACT GTT TAT GTC TAT GTT			567
Cys Ser Tyr Arg Asp Val Asp Ile Ala Ser Thr Val Tyr Val Tyr Val	90	95	100
CGA GAT TAC AGA TCA CCA TTC ATC GCC TCT GTC AGT GAC GAC CAT GGC			615
Arg Asp Tyr Arg Ser Pro Phe Ile Ala Ser Val Ser Asp Gln His Gly	105	110	115
ATC GTG TAC ATC ACC GAG AAC AAG AAC AAA ACT GTG GTG ATC CCC TGC			663
Ile Val Tyr Ile Thr Glu Asn Lys Asn Lys Thr Val Val Ile Pro Cys	120	125	130
CGA GGG TCG ATT TCA AAC CTC AAT GTG TCT CTT TGC GCT AGG TAT CCA			711
Arg Gly Ser Ile Ser Asn Leu Asn Val Ser Leu Cys Ala Arg Tyr Pro	135	140	145
GAA AAG AGA TTT GTT CCG GAT GGA AAC AGA ATT TCC TGG GAC AGC GAG			759
Glu Lys Arg Phe Val Pro Asp Gly Asn Arg Ile Ser Trp Asp Ser Glu	150	155	160
ATA GGC TTT ACT CTC CCC AGT TAC ATG ATC AGC TAT GCC GGC ATG GTC			807
Ile Gly Phe Thr Leu Pro Ser Tyr Met Ile Ser Tyr Ala Gly Met Val	170	175	180
TTC TGT GAG GCA AAG ATC AAT GAT GAA ACC TAT CAG TCT ATC ATG TAC			855
Phe Cys Glu Ala Lys Ile Asn Asp Glu Thr Tyr Gln Ser Ile Met Tyr	185	190	195
ATA GTT GTG GTT GTA GGA TAT AGG ATT TAT GAT GTG ATT CTG AGC CCC			903
Ile Val Val Val Val Gly Tyr Arg Ile Tyr Asp Val Ile Leu Ser Pro	200	205	210
CCG CAT GAA ATT GAG CTA TCT GCC GGA GAA AAA CTT GTC TTA AAT TGT			951
Pro His Glu Ile Glu Leu Ser Ala Gly Glu Lys Leu Val Leu Asn Cys	215	220	225

ACA GCG AGA ACA GAG CTC AAT GTG GGG CTT GAT TTC ACC TGG CAC TCT 999
 Thr Ala Arg Thr Glu Leu Asn Val Gly Leu Asp Phe Thr Trp His Ser 245
 230

CCA CCT TCA AAG TCT CAT CAT AAG AAG ATT GTA AAC CGG GAT GTG AAA 1047
 Pro Pro Ser Lys Ser His His Lys Lys Ile Val Asn Arg Asp Val Lys 260
 250

CCC TTT CCT GGG ACT GTG GCG AAG ATG TTT TTG AGC ACC TTG ACA ATA 1095
 Pro Phe Pro Gly Thr Val Ala Lys Met Phe Leu Ser Thr Leu Thr Ile 275
 265

GAA AGT GTG ACC AAG AGT GAC CAA GGG GAA TAC ACC TGT GTA GCG TCC 1143
 Glu Ser Val Thr Lys Ser Asp Gln Gly Glu Tyr Thr Cys Val Ala Ser 290
 280

AGT GGA CGG ATG ATC AAG AGA AAT AGA ACA TTT GTC CGA GTT CAC ACA 1191
 Ser Gly Arg Met Ile Lys Arg Asn Arg Thr Phe Val Arg Val His Thr 305
 295

AAG CCT TTT ATT GCT TTC GGT AGT GGG ATG AAA TCT TTG GTG GAA GCC 1239
 Lys Pro Phe Ile Ala Phe Phe Gly Ser Gly Met Lys Ser Leu Val Glu Ala 325
 310

ACA GTG GGC AGT CAA GTC CGA ATC CCT GTG AAG TAT CTC AGT TAC CCA 1287
 Thr Val Gly Ser Gln Val Arg Ile Pro Val Lys Tyr Leu Ser Tyr Pro 340
 330

GCT CCT GAT ATC AAA TGG TAC AGA AAT GGA AGG CCC ATT GAG TCC AAC 1335
 Ala Pro Asp Ile Lys Trp Tyr Arg Asn Gly Arg Pro Ile Glu Ser Asn 355
 345

TAC ACA ATG ATT GTT GGC GAT GAA CTC ACC ATC ATG GAA GTG ACT GAA 1383
 Tyr Thr Met Ile val Gly Asp Glu Leu Thr Ile Met Glu Val Thr Glu 370
 360

AGA GAT GCA GGA AAC TAC ACG GTC ATC CTC ACC AAC CCC ATT TCA ATG 1431

Arg Asp Ala Gly Asn Tyr Thr Val Ile Leu Thr Asn Pro Ile Ser Met
 375 380 385
 GAG AAA CAG AGC CAC ATG GTC TCT CTG GTT GTG AAT GTC CCA CCC CAG
 1479
 Glu Lys Gln Ser His Met Val Ser Leu Val Val Asn Val Pro Pro Gln
 390 395 400 405
 ATC GGT GAG AAA GCC TTG ATC TCG CCT ATG GAT TCC TAC CAG TAT GGG
 1527
 Ile Gly Glu Lys Ala Leu Ile Ser Pro Met Asp Ser Tyr Gln Tyr Gly
 410 415 420
 ACC ATG CAG ACA TTG ACA TGC ACA GTC TAC GCC AAC CCT CCC CTG CAC
 1575
 Thr Met Gln Thr Leu Thr Cys Thr Val Tyr Ala Asn Pro Pro Leu His
 425 430 435
 CAC ATC CAG TGG TAC TGG CAG CTA GAA GAA GCC TGC TCC TAC AGA CCC
 1623
 His Ile Gln Trp Tyr Trp Trp Gln Leu Glu Glu Ala Cys Ser Tyr Arg Pro
 440 445 450
 GGC CAA ACA AGC CCG TAT GCT TGT AAA GAA TGG AGA CAC GTG GAG GAT
 1671
 Gly Gln Thr Ser Pro Tyr Ala Cys Lys Glu Trp Arg His Val Glu Asp
 455 460 465
 TTC CAG GGG GGA AAC AAG ATC GAA GTC ACC AAA AAC CAA TAT GCC CTG
 1719
 Phe Gln Gly Gly Asn Lys Ile Glu Val Thr Lys Asn Gln Tyr Ala Leu
 470 475 480 485
 ATT GAA GGA AAA AAC AAA ACT GTA AGT ACG CTG GTC ATC CAA GCT GCC
 1767
 Ile Glu Gly Lys Asn Lys Thr Val Ser Thr Leu Val Ile Gln Ala Ala
 490 495 500
 AAC GTG TCA GCG TTG TAC AAA TGT GAA GCC ATC AAC AAA GCG GGA CGA
 1815
 Asn Val Ser Ala Leu Tyr Lys Cys Glu Ala Ile Asn Lys Ala Gly Arg
 505 510 515
 GGA GAG AGG GTC ATC TCC TTC CAT GTG ATC AGG GGT CCT GAA ATT ACT
 1863
 Gly Glu Arg Val Ile Ser Phe His Val Ile Arg Gly Pro Glu Ile Thr

520	525	530	1911
GTG CAA CCT GCT GCC CAG CCA ACT GAG CAG GAG AGT GTG TCC CTG TTG			
Val Gln Pro Ala Ala Gln Pro Thr Glu Gln Glu Ser Val Ser Leu Leu			
535	540	545	
TGC ACT GCA GAC AGA AAT ACG TTT GAG AAC CTC ACG TGG TAC AAG CTT			1959
Cys Thr Ala Asp Arg Asn Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu			
550	555	560	565
GGC TCA CAG GCA ACA TCG GTC CAC ATG GGC GAA TCA CTC ACA CCA GTT			2007
Gly Ser Gln Ala Thr Ser Val His Met Gly Glu Ser Leu Thr Pro Val			
570	575	580	
TGC AAG AAC TTG GAT GCT CTT TGG AAA CTG AAT GGC ACC ATG TTT TCT			2055
Cys Lys Asn Leu Asp Ala Leu Trp Lys Leu Asn Gly Thr Met Phe Ser			
585	590	595	
AAC AGC ACA AAT GAC ATC TTG ATT GTG GCA TTT CAG AAT GCC TCT CTG			2103
Asn Ser Thr Asn Asp Ile Leu Ile Val Ala Phe Gln Asn Ala Ser Leu			
600	605	610	
CAG GAC CAA GGC GAC TAT GTT TGC TCT GCT CAA GAT AAG AAG ACC AAG			2151
Gln Asp Gln Gly Asp Tyr Val Cys Ser Ala Gln Asp Lys Lys Thr Lys			
615	620	625	
AAA AGA CAT TGC CTG GTC AAA CAG CTC ATC ATC CTA GAG CGC ATG GCA			2199
Lys Arg His Cys Leu Val Lys Gln Leu Ile Ile Leu Glu Arg Met Ala			
630	635	640	645
CCC ATG ATC ACC GGA AAT CTG GAG AAT CAG ACA ACA ACC ATT GGC GAG			2247
Pro Met Ile Thr Gly Asn Leu Glu Asn Gln Thr Thr Thr Ile Gly Glu			
650	655	660	
ACC ATT GAA GTG ACT TGC CCA GCA TCT GGA AAT CCT ACC CCA CAC ATT			2295
Thr Ile Glu Val Thr Cys Pro Ala Ser Gly Asn Pro Thr Pro His Ile			
665	670	675	

2343
 ACA TGG TTC AAA GAC AAC GAG ACC CTG GTA GAA GAT TCA GGC ATT GTA
 Thr Trp Phe Lys Asp Asn Glu Thr Leu Val Glu Asp Ser Gly Ile Val
 680 685 690

2391
 CTG AGA GAT GGG AAC CGG AAC CTG ACT ATC CGC AGG GTG AGG AAG GAG
 Leu Arg Asp Gly Asn Arg Asn Leu Thr Ile Arg Arg Val Arg Lys Glu
 695 700 705

2439
 GAT GGA GGC CTC TAC ACC TGC CAG GCC TGC AAT GTC CTT GGC TGT GCA
 Asp Gly Gly Leu Tyr Thr Cys Gln Ala Cys Asn Val Leu Gly Cys Ala
 710 715 720 725

2487
 AGA GCG GAG ACG CTC TTC ATA ATA GAA GGT GCC CAG GAA AAG ACC AAC
 Arg Ala Glu Thr Leu Phe Ile Ile Glu Gly Ala Gln Glu Lys Thr Asn
 730 735 740

2535
 TTG GAA GTC ATT ATC CTC GTC GGC ACT GCA GTG ATT GCC ATG TTC TTC
 Leu Glu Val Ile Ile Leu Val Gly Thr Ala Val Ile Ala Met Phe Phe
 745 750 755

2583
 TGG CTC CTT CTT GTC ATT CTC GTA CGG ACC GTT AAG CGG GCC AAT GAA
 Trp Leu Leu Leu Val Ile Leu Val Arg Thr Val Lys Arg Ala Asn Glu
 760 765 770

2631
 GGG GAA CTG AAG ACA GGC TAC TTG TCT ATT GTC ATG GAT CCA GAT GAA
 Gly Glu Leu Lys Thr Gly Tyr Leu Ser Ile Val Met Asp Pro Asp Glu
 775 780 785

2679
 TTG CCC TTG GAT GAG CGC TGT GAA CGC TTG CCT TAT GAT GCC AGC AAG
 Leu Pro Leu Asp Glu Arg Cys Glu Arg Leu Pro Tyr Asp Ala Ser Lys
 790 795 800 805

2727
 TGG GAA TTC CCC AGG GAC CGG CTG AAA CTA GGA AAA CCT CTT GGC CGC
 Trp Glu Phe Pro Arg Asp Arg Leu Lys Leu Gly Lys Pro Leu Gly Arg
 810 815 820

2775
 GGT GCC TTC GGC CAA GTG ATT GAG GCA GAC GCT TTT GGA ATT GAC AAG

Gly Ala Phe Gly Gln Val Ile Glu Ala Asp Ala Phe Gly Ile Asp Lys
 825 830 835
 2823
 ACA GCG ACT TGC AAA ACA GTA GCC GTC AAG ATG TTG AAA GAA GGA GCA
 Thr Ala Thr Cys Lys Thr Val Ala Val 845
 840
 2871
 ACA CAC AGC GAG CAT CGA GCC CTC ATG TCT GAA CTC AAG ATC CTC ATC
 Thr His Ser Glu His Arg Ala Leu Met Ser Glu Leu Lys Ile Leu Ile
 855 860 865
 2919
 CAC ATT GGT CAC CAT CTC AAT GTG GTG AAC CTC CTA GGC GCC TGC ACC
 His Ile Gly His His Leu Asn Val Val Asn Leu Leu Gly Ala Cys Thr
 870 875 880 885
 2967
 AAG CCG GGA GGG CCT CTC ATG GTG ATT GTG GAA TTC TCG AAG TTT GGA
 Lys Pro Gly Gly Pro Leu Met Val Ile Val Glu Phe Ser Lys Phe Gly
 890 895 900
 3015
 AAC CTA TCA ACT TAC TTA CGG GGC AAG AGA AAT GAA TTT GTT CCC TAT
 Asn Leu Ser Thr Tyr Leu Leu Arg Gly Lys Arg Asn Glu Phe Val Pro Tyr
 905 910 915
 3063
 AAG AGC AAA GGG GCA CGC TTC CGC CAG GGC AAG GAC TAC GTT GGG GAG
 Lys Ser Lys Gly Ala Arg Phe Arg Gln Gly Lys Asp Tyr val Gly Glu
 920 925 930
 3111
 CTC TCC GTG GAT CTG AAA AGA CGC TTG GAC AGC ATC ACC AGC AGC CAG
 Leu Ser Val Asp Leu Lys Arg Arg Leu Asp Ser Ile Thr Ser Ser Gln
 935 940 945
 3159
 AGC TCT GCC AGC TCA GGC TTT GTT GAG GAG AAA TCG CTC AGT GAT GTA
 Ser Ser Ala Ser Ser Gly Phe Val Glu Glu Lys Ser Leu Ser Asp Val
 950 955 960 965
 3207
 GAG GAA GAA GAA GCT TCT GAA GAA CTG TAC AAG GAC TTC CTG ACC TTG
 Glu Glu Glu Glu Ala Ser Glu Glu Leu Tyr Lys Asp Phe Leu Thr Leu

970 975 980
 GAG CAT CTC ATC TGT TAC AGC TTC CAA GTG GCT AAG GGC ATG GAG TTC 3255
 Glu His Leu Ile Cys Tyr Ser Phe 985
 990
 TGT GCA TCA AGG AAG TGT ATC CAC AGG GAC CTG GCA GCA CGA AAC ATT 3303
 Leu Ala Ser Arg Lys Cys Ile His Arg Asp Leu Ala Ala Arg Asn Ile
 1000 1005 1010
 CTC CTA TCG GAG AAG AAT GTG GTT AAG ATC TGT GAC TTC GGC TTG GCC 3351
 Leu Leu Ser Glu Lys Asn Val Val Lys Ile Cys Asp Phe Gly Leu Ala
 1015 1020 1025
 CGG GAC ATT TAT AAA GAC CCG GAT TAT GTC AGA AAA GGA GAT GCC CGA 3399
 Arg Asp Ile Tyr Lys Asp Pro Asp Tyr Val Arg Lys Gly Asp Ala Arg
 1030 1035 1040 1045
 CTC CCT TTG AAG TGG ATG GCC CCG GAA ACC ATT TTT GAC AGA GTA TAC 3447
 Leu Pro Leu Lys Trp Met Ala Pro Glu Thr Ile Phe Asp Arg Val Tyr
 1050 1055 1060
 ACA ATT CAG AGC GAT GTG TGG TCT TTC GGT GTG TTG CTC TGG GAA ATA 3495
 Thr Ile Gln Ser Asp Val Trp Ser Phe Gly Val Leu Leu Trp Glu Ile
 1065 1070 1075
 TTT TCC TTA GGT GCC TCC CCA TAC CCT GGG GTC AAG ATT GAT GAA GAA 3543
 Phe Ser Leu Gly Ala Ser Pro Tyr Pro Gly Val Lys Ile Asp Glu Glu
 1080 1085 1090
 TTT TGT AGG AGA TTG AAA GAA GGA ACT AGA ATG CGG GCT CCT GAC TAC 3591
 Phe Cys Arg Arg Leu Lys Glu Gly Thr Arg Met Arg Ala Pro Asp Tyr
 1095 1100 1105
 ACT ACC CCA GAA ATG TAC CAG ACC ATG CTG GAC TGC TGG CAT GAG GAC 3639
 Thr Thr Pro Glu Met Tyr Gln Thr Met Leu Asp Cys Trp His Glu Asp
 1110 1115 1120 1125

CCC AAC CAG AGA CCC TCG TTT TCA GAG TTG GTG GAG CAT TTG GGA AAC 3687
 Pro Asn Gln Arg Pro Ser Phe Ser Glu Leu Val Glu His Leu Gly Asn 1140
 1130 1135
 CTC CTG CAA GCA AAT GCG CAG CAG GAT GGC AAA GAC TAT ATT GTT CTT 3735
 Leu Leu Gln Ala Asn Ala Gln Gln Asp Gly Lys Asp Tyr Ile Val Leu 1155
 1145 1150
 CCA ATG TCA GAG ACA CTG AGC ATG GAA GAG GAT TCT GGA CTC TCC CTG 3783
 Pro Met Ser Glu Thr Leu Ser Met Glu Glu Asp Ser Gly Leu Ser Leu 1170
 1160 1165
 CCT ACC TCA CCT GTT TCC TGT ATG GAG GAA GAG GTG TGC GAC CCC 3831
 Pro Thr Ser Pro Val Ser Cys Met Glu Glu Glu Val Cys Asp Pro 1185
 1175
 AAA TTC CAT TAT GAC AAC ACA GCA GGA ATC AGT CAT TAT CTC CAG AAC 3879
 Lys Phe His Tyr Asp Asn Thr Ala Gly Ile Ser His Tyr Leu Gln Asn 1205
 1190 1195
 AGT AAG CGA AAG AGC CGG CCA GTG AGT GTA AAA ACA TTT GAA GAT ATC 3927
 Ser Lys Arg Lys Ser Arg Pro Val Ser Val Lys Thr Phe Glu Asp Ile 1220
 1210
 CCA TTG GAG GAA CCA GAA GTA AAA GTG ATC CCA GAT GAC AGC CAG ACA 3975
 Pro Leu Glu Glu Pro Glu Val Lys Val Ile Pro Asp Asp Ser Gln Thr 1235
 1225
 GAC AGT GGG ATG GTC CTT GCA TCA GAA GAG CTG AAA ACT CTG GAA GAC 4023
 Asp Ser Gly Met Val Leu Ala Ser Glu Glu Leu Lys Thr Leu Glu Asp 1250
 1240 1245
 AGG AAC AAA TTA TCT CCA TCT TTT GGT GGA ATG ATG CCC AGT AAA AGC 4071
 Arg Asn Lys Leu Ser Pro Ser Phe Gly Gly Met Met Pro Ser Lys Ser 1265
 1255 1260
 AGG GAG TCT GTG GCC TCG GAA GGC TCC AAC CAG ACC AGT GGC TAC CAG 4119

Arg Glu Ser Val Ala Ser Glu Gly Ser Asn Gln Thr Ser Gly Tyr Gln 1285
 1270 1275
 TCT GGG TAT CAC TCA GAT GAC ACA GAC ACC ACC GTG TAC TCC AGC GAC 4167
 Ser Gly Tyr His Ser Asp Asp Thr Asp Thr Thr Val Tyr Ser Ser Asp 1300
 1290 1295
 GAG GCA GGA CTT TTA AAG ATG GTG GAT GCT GCA GTT CAC GCT GAC TCA 4215
 Glu Ala Gly Leu Lys Met Val Asp Ala Ala Val His Ala Asp Ser 1315
 1305 1310
 GGG ACC ACA CTG CAG CTC ACC TCC TGT TTA AAT GGA AGT GGT CCT GTC 4263
 Gly Thr Thr Leu Gln Leu Thr Ser Cys Leu Asn Gly Ser Gly Pro Val 1330
 1320 1325
 CCG GCT CCG CCC CCA ACT CCT GGA AAT CAC GAG AGA GGT GCT TAGATTTTCA 4318
 Pro Ala Pro Pro Thr Pro Gly Asn His Glu Arg Gly Ala Ala 1345
 1335 1340
 AGTGTGTGTC TTTCACCAC CCGGAAGTAG CCACATTGA TTTTCATTTT TGGAGGAGGG 4378
 ACCTCAGACT GCAAGGAGCT TGTCCCTCAGG GCATTTCAG AGAAGATGCC CATGACCCAA 4438
 GAATGTGTTG ACTCTACTCT CTTTCCATT CATTTAAAAG TCCTATATAA TGTGCCCTGC 4498
 TGTGGTCTCA CTACCAGTTA AAGCAAAAGA CTTTCAAACA CGTGGACTCT GTCCTCCAAG 4558
 AAGTGGCAAC GGCACCTCTG TGAAACTGGA TCGAATGGGC AATGCTTTGT GTGTTGAGGA 4618
 TGGGTGAGAT GTCCCAGGGC CGAGTCTGTC TACCTTGGAG GCTTTGTGGA GGATGCGGCT 4678
 ATGAGCCAAG TGTTAAGTGT GGGATGTGGA CTGGGAGGAA GGAAGGCCGA AGCCGTCCGG 4738
 AGAGCGGTTG GAGCCTGCAG ATGCATTGTG CTGGCTCTGG TGGAGGTGGG CTTGTGGCCT 4798
 GTCAGGAAAC GCAAAGGCGG CCGGCAGGGT TTGGTTTTGG AAGGTTTGG TGCTCTTCAC 4858

AGTCGGGTTA CAGGCGAGTT CCCTGTGGCG TTTCCTACTC CTAATGAGAG TTCCTTCCGG 4918
 ACTCTTACGT GTCTCCTGGC CTGGCCCCCAG GAAGGAAATG ATGCAGCTTG CTCCTTCCCTC 4978
 ATCTCTCAGG CTGTGCCTTA ATTCAGAACA CCAAAAGAGA GGAACGTCGG CAGAGGCTCC 5038
 TGACGGGGCC GAAGAATTGT GAGAACAGAA CAGAAACTCA GGGTTTCTGC TGGGTGGAGA 5098
 CCCACGTGGC GCCCTGGTGG CAGGCTCTGAG GGTTCTCTGT CAAGTGGCGG TAAAGGCTCA 5158
 GGCTGGTGTT CTTCCTCTAT CTCCACTCCT GTCAGGCCCC CAAGTCCTCA GTATTTTAGC 5218
 TTTGTGGCTT CCTGATGGCA GAAAAATCTT AATTGGTTGG TTTGCTCTCC AGATAATCAC 5278
 TAGCCAGATT TCGAAATTAC TTTTtagccg AGGTTATGAT AACATCTACT GTATCCTTTA 5338
 GAATTTTAAC CTATAAAACT ATGTCTACTG GTTCTGCCT GTGTGCTTAT GTTAAAAAAA 5398
 AAAAAAAA 5406

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1367 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Glu Ser Lys Gly Leu Leu Ala Val Ala Leu Trp Phe Cys Val Glu
 -19 -15 -10 -5

Thr Arg Ala Ala Ser Val Gly Leu Pro Gly Asp Phe Leu His Pro Pro
 1 5 10

Lys Leu Ser Thr Gln Lys Asp Ile Leu Thr Ile Leu Ala Asn Thr Thr
 15 20 25
 Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
 30 35 40 45
 Asn Ala Gln Arg Asp Ser Glu Glu Arg Val Leu Val Thr Glu Cys Gly
 50 55 60
 Gly Gly Asp Ser Ile Phe Cys Lys Thr Leu Thr Ile Pro Arg Val Val
 65 70 75
 Gly Asn Asp Thr Gly Ala Tyr Lys Cys Ser Tyr Arg Asp Val Asp Ile
 80 85 90
 Ala Ser Thr val Tyr val Tyr val Arg Asp Tyr Arg Ser Pro Phe Ile
 95 100 105
 Ala Ser val Ser Asp Gln His Gly Ile val Tyr Ile Thr Glu Asn Lys
 110 115 120 125
 Asn Lys Thr Val Ile Pro Cys Arg Gly Ser Ile Ser Asn Leu Asn
 130 135 140
 Val Ser Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly
 145 150 155
 Asn Arg Ile Ser Trp Asp Ser Glu Ile Gly Phe Thr Leu Pro Ser Tyr
 160 165 170
 Met Ile Ser Tyr Ala Gly Met val Phe Cys Glu Ala Lys Ile Asn Asp
 175 180 185
 Glu Thr Tyr Gln Ser Ile Met Tyr Ile val Val val Gly Tyr Arg
 190 195 200 205
 Ile Tyr Asp val Ile Leu Ser Pro Pro His Glu Ile Glu Leu Ser Ala

	210	215	220
Gly Glu Lys	Leu Val	Thr Ala Arg Thr Glu	Leu Asn Val
225	230		235
Gly Leu Asp Phe Thr Trp His Ser Pro Pro Ser Lys Ser His His Lys			
240	245		250
Lys Ile Val Asn Arg Asp Val Lys Pro Phe Pro Gly Thr Val Ala Lys			
255	260		265
Met Phe Leu Ser Thr Leu Thr Ile Glu Ser Val Thr Lys Ser Asp Gln			
270	275	280	285
Gly Glu Tyr Thr Cys Val Ala Ser Ser Gly Arg Met Ile Lys Arg Asn			
290	295		300
Arg Thr Phe Val Arg Val His Thr Lys Pro Phe Ile Ala Phe Gly Ser			
305	310		315
Gly Met Lys Ser Leu Val Glu Ala Thr Val Gly Ser Gln Val Arg Ile			
320	325		330
Pro Val Lys Tyr Leu Ser Tyr Pro Ala Pro Asp Ile Lys Trp Tyr Arg			
335	340	345	
Asn Gly Arg Pro Ile Glu Ser Asn Tyr Thr Met Ile Val Gly Asp Glu			
350	355	360	365
Leu Thr Ile Met Glu Val Thr Glu Arg Asp Ala Gly Asn Tyr Thr Val			
370	375		380
Ile Leu Thr Asn Pro Ile Ser Met Glu Lys Gln Ser His Met Val Ser			
385	390		395
Leu Val Val Asn Val Pro Pro Gln Ile Gly Glu Lys Ala Leu Ile Ser			
400	405		410

Pro Met Asp Ser Tyr Gln Tyr Gly Thr Met Gln Thr Leu Thr Cys Thr
 415 420 425
 Val Tyr Ala Asn Pro Pro Leu His His Ile Gln Trp Tyr Trp Gln Leu
 430 435 440 445
 Glu Glu Ala Cys Ser Tyr Arg Pro Gly Gln Thr Ser Pro Tyr Ala Cys
 450 455 460
 Lys Glu Trp Arg His Val Glu Asp Phe Gln Gly Gly Asn Lys Ile Glu
 465 470 475
 Val Thr Lys Asn Gln Tyr Ala Leu Ile Glu Gly Lys Asn Lys Thr Val
 480 485 490
 Ser Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu Tyr Lys Cys
 495 500 505
 Glu Ala Ile Asn Lys Ala Gly Arg Gly Glu Arg Val Ile Ser Phe His
 510 515 520 525
 Val Ile Arg Gly Pro Glu Ile Thr Val Gln Pro Ala Ala Gln Pro Thr
 530 535 540
 Glu Gln Glu Ser Val Ser Leu Leu Cys Thr Ala Asp Arg Asn Thr Phe
 545 550 555
 Glu Asn Leu Thr Trp Tyr Lys Leu Gly Ser Gln Ala Thr Ser Val His
 560 565 570
 Met Gly Glu Ser Leu Thr Pro Val Cys Lys Asn Leu Asp Ala Leu Trp
 575 580 585
 Lys Leu Asn Gly Thr Met Phe Ser Asn Ser Thr Asn Asp Ile Leu Ile
 590 595 600 605
 Val Ala Phe Gln Asn Ala Ser Leu Gln Asp Gln Gly Asp Tyr Val Cys

610	615	620
Ser Ala Gln Asp Lys Lys Thr Lys Lys Arg His Cys Leu Val Lys Gln		
625	630	635
Leu Ile Ile Leu Glu Arg Met Ala Pro Met Ile Thr Gly Asn Leu Glu		
640	645	650
Asn Gln Thr Thr Thr Ile Gly Glu Thr Ile Glu Val Thr Cys Pro Ala		
655	660	665
Ser Gly Asn Pro Thr Pro His Ile Thr Trp Phe Lys Asp Asn Glu Thr		
670	675	680
Leu Val Glu Asp Ser Gly Ile Val Leu Arg Asp Gly Asn Arg Asn Leu		
690	695	700
Thr Ile Arg Arg Val Arg Lys Glu Asp Gly Gly Leu Tyr Thr Cys Gln		
705	710	715
Ala Cys Asn Val Leu Gly Cys Ala Arg Ala Glu Thr Leu Phe Ile Ile		
720	725	730
Glu Gly Ala Gln Glu Lys Thr Asn Leu Glu Val Ile Ile Leu Val Gly		
735	740	745
Thr Ala Val Ile Ala Met Phe Phe Trp Leu Leu Val Ile Leu Val		
750	755	760
Arg Thr Val Lys Arg Ala Asn Glu Gly Glu Leu Lys Thr Gly Tyr Leu		
765	770	775
Ser Ile Val Met Asp Pro Asp Glu Leu Pro Leu Asp Glu Arg Cys Glu		
785	790	795
Arg Leu Pro Tyr Asp Ala Ser Lys Trp Glu Phe Pro Arg Asp Arg Leu		
800	805	810

Lys Leu Gly Lys Pro Leu Gly Arg Gly Ala Phe Gly Gln Val Ile Glu
 815 820 825
 Ala Asp Ala Phe Gly Ile Asp Lys Thr Ala Thr Cys Lys Thr Val Ala
 830 835 840 845
 Val Lys Met Leu Lys Glu Gly Ala Thr His Ser Glu His Arg Ala Leu
 850 855 860
 Met Ser Glu Leu Lys Ile Leu Ile His Ile Gly His His Leu Asn Val
 865 870 875
 Val Asn Leu Leu Gly Ala Cys Thr Lys Pro Gly Gly Pro Leu Met Val
 880 885 890
 Ile Val Glu Phe Ser Lys Phe Gly Asn Leu Ser Thr Tyr Leu Arg Gly
 895 900 905
 Lys Arg Asn Glu Phe Val Pro Tyr Lys Ser Lys Gly Ala Arg Phe Arg
 910 915 920 925
 Gln Gly Lys Asp Tyr Val Gly Glu Leu Ser Val Asp Leu Lys Arg Arg
 930 935 940
 Leu Asp Ser Ile Thr Ser Ser Gln Ser Ser Ala Ser Ser Gly Phe Val
 945 950 955
 Glu Glu Lys Ser Leu Ser Asp Val Glu Glu Glu Ala Ser Glu Glu
 960 965 970
 Leu Tyr Lys Asp Phe Leu Thr Leu Glu His Leu Ile Cys Tyr Ser Phe
 975 980 985
 Gln Val Ala Lys Gly Met Glu Phe Leu Ala Ser Arg Lys Cys Ile His
 990 995 1000 1005
 Arg Asp Leu Ala Ala Arg Asn Ile Leu Leu Ser Glu Lys Asn Val Val

1010	1015	1020
Lys ile Cys Asp Phe Gly Leu Ala Arg Asp ile Tyr Lys Asp Pro Asp		
1025	1030	1035
Tyr val Arg Lys Gly Asp Ala Arg Leu Pro Leu Lys Trp Met Ala Pro		
1040	1045	1050
Glu Thr ile Phe Asp Arg val Tyr Thr ile Gln Ser Asp val Trp Ser		
1055	1060	1065
Phe Gly val Leu Leu Trp Glu ile Phe Ser Leu Gly Ala Ser Pro Tyr		
1070	1075	1080
Pro Gly val Lys ile Asp Glu Glu Phe Cys Arg Arg Leu Lys Glu Gly		
1090	1095	1100
Thr Arg Met Arg Ala Pro Asp Tyr Thr Thr Pro Glu Met Tyr Gln Thr		
1105	1110	1115
Met Leu Asp Cys Trp His Glu Asp Pro Asn Gln Arg Pro Ser Phe Ser		
1120	1125	1130
Glu Leu val Glu His Leu Gly Asn Leu Leu Gln Ala Asn Ala Gln Gln		
1135	1140	1145
Asp Gly Lys Asp Tyr ile val Leu Pro Met Ser Glu Thr Leu Ser Met		
1150	1155	1160
Glu Glu Asp Ser Gly Leu Ser Leu Pro Thr Ser Pro val Ser Cys Met		
1170	1175	1180
Glu Glu Glu Glu val Cys Asp Pro Lys Phe His Tyr Asp Asn Thr Ala		
1185	1190	1195
Gly ile Ser His Tyr Leu Gln Asn Ser Lys Arg Lys Ser Arg Pro val		
1200	1205	1210

Ser Val Lys Thr Phe Glu Asp Ile Pro Leu Glu Pro Glu Val Lys
 1215 1220
 Val Ile Pro Asp Asp Ser Gln Thr Asp Ser Gly Met Val Leu Ala Ser
 1230 1235 1240 1245
 Glu Glu Leu Lys Thr Leu Glu Asp Arg Asn Lys Leu Ser Pro Ser Phe
 1250 1255 1260
 Gly Gly Met Met Pro Ser Lys Ser Arg Glu Ser Val Ala Ser Glu Gly
 1265 1270 1275
 Ser Asn Gln Thr Ser Gly Tyr Gln Ser Gly Tyr His Ser Asp Asp Thr
 1280 1285 1290
 Asp Thr Thr Val Tyr Ser Ser Asp Glu Ala Gly Leu Leu Lys Met Val
 1295 1300 1305
 Asp Ala Ala Val His Ala Asp Ser Gly Thr Thr Leu Gln Leu Thr Ser
 1310 1315 1320 1325
 Cys Leu Asn Gly Ser Gly Pro Val Pro Ala Pro Pro Thr Pro Gly
 1330 1335 1340
 Asn His Glu Arg Gly Ala Ala
 1345

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 96 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AATTCGTCGA CTTTCTGTCA CCATGAGTGC ACTTCTGATC CTAGCCCTTG TGGGAGCTGC 60

TGTTGCTGAC TACAAAAGATG ATGATGACAA GATCTA 96

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 96 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AGCTTAGATC TTGTCATCAT CATCTTTGTA GTCAGCAACA GCAGCTCCCA CAGAGGCTAG 60

GATCAGAAAGT GCACTCATGG TGACAGAAAG TCGACG 96

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGAGAAGATC TCAAACCAAG ACCTGCCTGT

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCAATGGCGG CCGCTCAGGA GATGTGTCT TGGA

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ala Gln Ser Leu Ser Phe Xaa Phe Thr Lys Phe Asp Leu Asp
1 5 10

CLAIMS

What is claimed is:

5

1. A protein that binds to the Flk2 receptor comprising the amino acid sequence AQSLSFXTKFDLD shown in SEQ. ID. NO. 11, wherein X is any amino acid.

10

GCGGCCTGGC	TACCGCGCGC	TCCGGAGGCC	ATG	CGG	GCG	TTG	GCG	CAG	CGC	AGC					
			Met	Arg	Ala	Leu	Ala	Gln	Arg	Ser					
			-27		-25										-20
GAC	CGG	CGG	CTG	CTG	CTG	CTT	GTT	GTT	TTG	TCA	GTA	ATG	ATT	CTT	GAG
Asp	Arg	Arg	Leu	Leu	Leu	Leu	Val	Val	Leu	Ser	Val	Met	Ile	Leu	Glu
			-15						-10					-5	
ACC	GTT	ACA	AAC	CAA	GAC	CTG	CCT	GTG	ATC	AAG	TGT	GTT	TTA	ATC	AGT
Thr	Val	Thr	Asn	Gln	Asp	Leu	Pro	Val	Ile	Lys	Cys	Val	Leu	Ile	Ser
			1				5					10			
CAT	GAG	AAC	AAT	GGC	TCA	TCA	GCG	GGA	AAG	CCA	TCA	TCG	TAC	CGA	ATG
His	Glu	Asn	Asn	Gly	Ser	Ser	Ala	Gly	Lys	Pro	Ser	Ser	Tyr	Arg	Met
	15					20					25				
GTG	CGA	GGA	TCC	CCA	GAA	GAC	CTC	CAG	TGT	ACC	CCG	AGG	CGC	CAG	AGT
Val	Arg	Gly	Ser	Pro	Glu	Asp	Leu	Gln	Cys	Thr	Pro	Arg	Arg	Gln	Ser
	30				35					40					45
GAA	GGG	ACG	GTA	TAT	GAA	GCG	GCC	ACC	GTG	GAG	GTG	GCC	GAG	TCT	GGG
Glu	Gly	Thr	Val	Tyr	Glu	Ala	Ala	Thr	Val	Glu	Val	Ala	Glu	Ser	Gly
				50					55					60	
TCC	ATC	ACC	CTG	CAA	GTG	CAG	CTC	GCC	ACC	CCA	GGG	GAC	CTT	TCC	TGC
Ser	Ile	Thr	Leu	Gln	Val	Gln	Leu	Ala	Thr	Pro	Gly	Asp	Leu	Ser	Cys
			65					70					75		
CTC	TGG	GTC	TTT	AAG	CAC	AGC	TCC	CTG	GGC	TGC	CAG	CCG	CAC	TTT	GAT
Leu	Trp	Val	Phe	Lys	His	Ser	Ser	Leu	Gly	Cys	Gln	Pro	His	Phe	Asp
		80					85					90			
TTA	CAA	AAC	AGA	GGA	ATC	GTT	TCC	ATG	GCC	ATC	TTG	AAC	GTG	ACA	GAG
Leu	Gln	Asn	Arg	Gly	Ile	Val	Ser	Met	Ala	Ile	Leu	Asn	Val	Thr	Glu
	95					100					105				
ACC	CAG	GCA	GGA	GAA	TAC	CTA	CTC	CAT	ATT	CAG	AGC	GAA	CGC	GCC	AAC
Thr	Gln	Ala	Gly	Glu	Tyr	Leu	Leu	His	Ile	Gln	Ser	Glu	Arg	Ala	Asn
					115					120					125
TAC	ACA	GTA	CTG	TTC	ACA	GTG	AAT	GTA	AGA	GAT	ACA	CAG	CTG	TAT	GTG
Tyr	Thr	Val	Leu	Phe	Thr	Val	Asn	Val	Arg	Asp	Thr	Gln	Leu	Tyr	Val
				130				135						140	
CTA	AGG	AGA	CCT	TAC	TTT	AGG	AAG	ATG	GAA	AAC	CAG	GAT	GCA	CTG	CTC
Leu	Arg	Arg	Pro	Tyr	Phe	Arg	Lys	Met	Glu	Asn	Gln	Asp	Ala	Leu	Leu
			145					150					155		

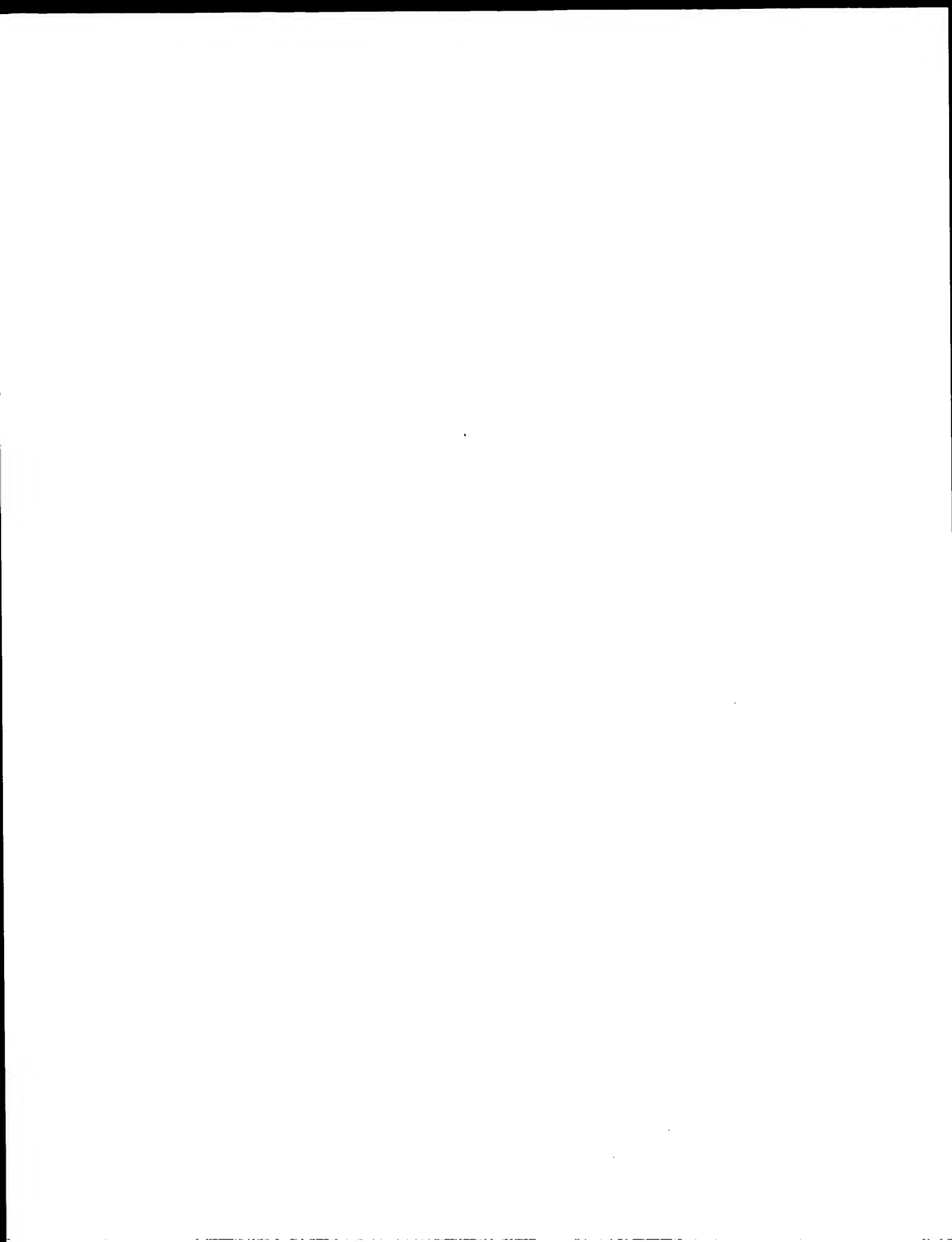


Fig. 1a.2

TGC	ATC	TCC	GAG	GGT	GTT	CCG	GAG	CCC	ACT	GTG	GAG	TGG	GTG	CTC	TGC
Cys	Ile	Ser	Glu	Gly	Val	Pro	Glu	Pro	Thr	Val	Glu	Trp	Val	Leu	Cys
		160					165					170			
AGC	TCC	CAC	AGG	GAA	AGC	TGT	AAA	GAA	GAA	GGC	CCT	GCT	GTT	GTC	AGA
Ser	Ser	His	Arg	Glu	Ser	Cys	Lys	Glu	Glu	Gly	Pro	Ala	Val	Val	Arg
	175					180					185				
AAG	GAG	GAA	AAG	GTA	CTT	CAT	GAG	TTG	TTC	GGA	ACA	GAC	ATC	AGA	TGC
Lys	Glu	Glu	Lys	Val	Leu	His	Glu	Leu	Phe	Gly	Thr	Asp	Ile	Arg	Cys
	190				195					200					205
TGT	GCT	AGA	AAT	GCA	CTG	GGC	CGC	GAA	TGC	ACC	AAG	CTG	TTC	ACC	ATA
Cys	Ala	Arg	Asn	Ala	Leu	Gly	Arg	Glu	Cys	Thr	Lys	Leu	Phe	Thr	Ile
				210					215					220	
GAT	CTA	AAC	CAG	GCT	CCT	CAG	AGC	ACA	CTG	CCC	CAG	TTA	TTC	CTG	AAA
Asp	Leu	Asn	Gln	Ala	Pro	Gln	Ser	Thr	Leu	Pro	Gln	Leu	Phe	Leu	Lys
			225					230					235		
GTG	GGG	GAA	CCC	TTG	TGG	ATC	AGG	TGT	AAG	GCC	ATC	CAT	GTG	AAC	CAT
Val	Gly	Glu	Pro	Leu	Trp	Ile	Arg	Cys	Lys	Ala	Ile	His	Val	Asn	His
		240					245					250			
GGA	TTC	GGG	CTC	ACC	TGG	GAG	CTG	GAA	GAC	AAA	GCC	CTG	GAG	GAG	GGC
Gly	Phe	Gly	Leu	Thr	Trp	Glu	Leu	Glu	Asp	Lys	Ala	Leu	Glu	Glu	Gly
	255					260					265				
AGC	TAC	TTT	GAG	ATG	AGT	ACC	TAC	TCC	ACA	AAC	AGG	ACC	ATG	ATT	CGG
Ser	Tyr	Phe	Glu	Met	Ser	Thr	Tyr	Ser	Thr	Asn	Arg	Thr	Met	Ile	Arg
	270				275					280					285
ATT	CTC	TTG	GCC	TTT	GTG	TCT	TCC	GTG	GGA	AGG	AAC	GAC	ACC	GGA	TAT
Ile	Leu	Leu	Ala	Phe	Val	Ser	Ser	Val	Gly	Arg	Asn	Asp	Thr	Gly	Tyr
				290					295					300	
TAC	ACC	TGC	TCT	TCC	TCA	AAG	CAC	CCC	AGC	CAG	TCA	GCG	TTG	GTG	ACC
Tyr	Thr	Cys	Ser	Ser	Ser	Lys	His	Pro	Ser	Gln	Ser	Ala	Leu	Val	Thr
			305					310					315		
ATC	CTA	GAA	AAA	GGG	TTT	ATA	AAC	GCT	ACC	AGC	TCG	CAA	GAA	GAG	TAT
Ile	Leu	Glu	Lys	Gly	Phe	Ile	Asn	Ala	Thr	Ser	Ser	Gln	Glu	Glu	Tyr
		320					325					330			
GAA	ATT	GAC	CCG	TAC	GAA	AAG	TTC	TGC	TTC	TCA	GTC	AGG	TTT	AAA	GCG
Glu	Ile	Asp	Pro	Tyr	Glu	Lys	Phe	Cys	Phe	Ser	Val	Arg	Phe	Lys	Ala
	335					340					345				

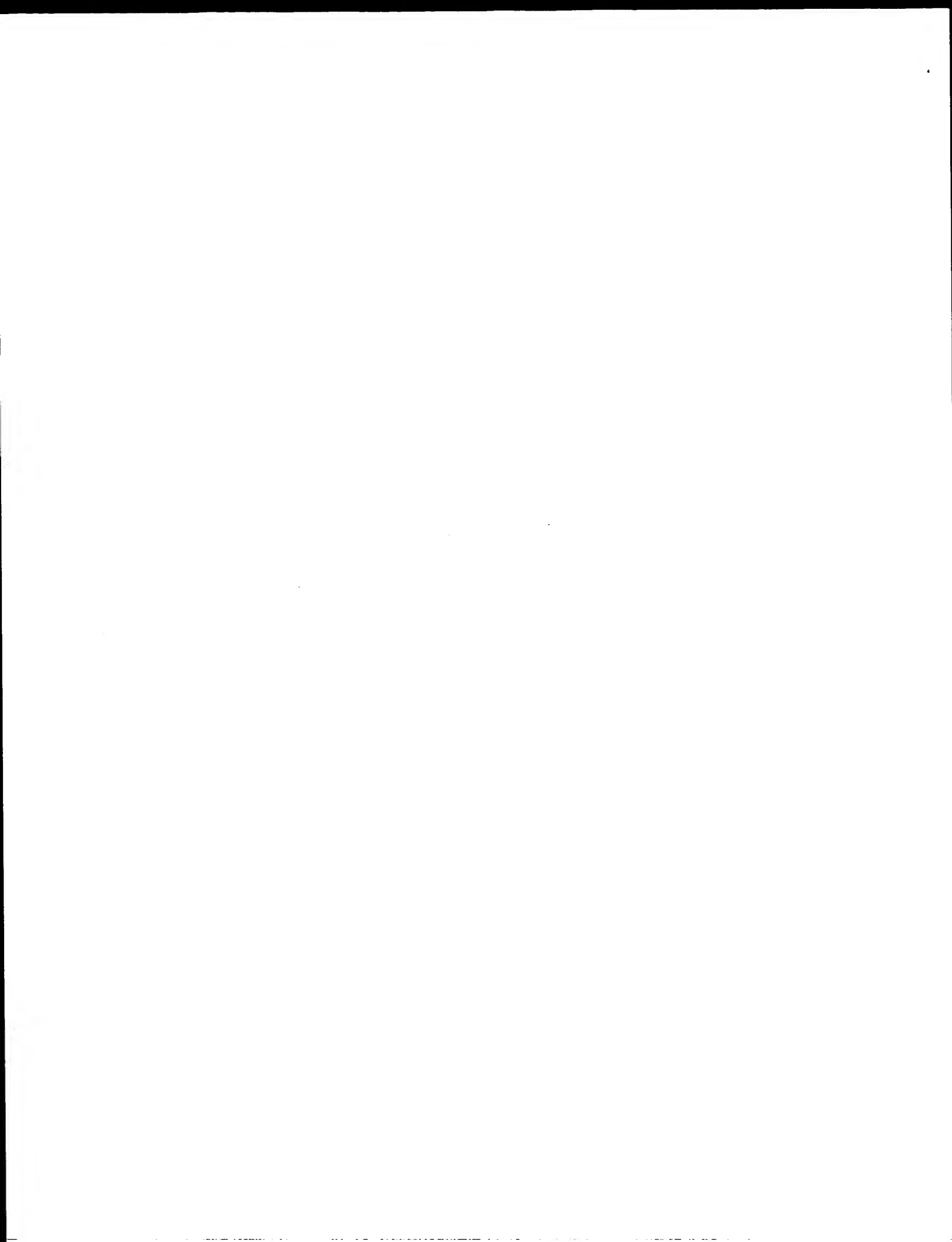


Fig. 1a.3

TAC	CCA	CGA	ATC	CGA	TGC	ACG	TGG	ATC	TTC	TCT	CAA	GCC	TCA	TTT	CCT
Tyr	Pro	Arg	Ile	Arg	Cys	Thr	Trp	Ile	Phe	Ser	Gln	Ala	Ser	Phe	Pro
350					355					360					365
TGT	GAA	CAG	AGA	GGC	CTG	GAG	GAT	GGG	TAC	AGC	ATA	TCT	AAA	TTT	TGC
Cys	Glu	Gln	Arg	Gly	Leu	Glu	Asp	Gly	Tyr	Ser	Ile	Ser	Lys	Phe	Cys
				370					375					380	
GAT	CAT	AAG	AAC	AAG	CCA	GGA	GAG	TAC	ATA	TTC	TAT	GCA	GAA	AAT	GAT
Asp	His	Lys	Asn	Lys	Pro	Gly	Glu	Tyr	Ile	Phe	Tyr	Ala	Glu	Asn	Asp
			385					390					395		
GAC	GCC	CAG	TTC	ACC	AAA	ATG	TTC	ACG	CTG	AAT	ATA	AGA	AAG	AAA	CCT
Asp	Ala	Gln	Phe	Thr	Lys	Met	Phe	Thr	Leu	Asn	Ile	Arg	Lys	Lys	Pro
		400					405					410			
CAA	GTG	CTA	GCA	AAT	GCC	TCA	GCC	AGC	CAG	GCG	TCC	TGT	TCC	TCT	GAT
Gln	Val	Leu	Ala	Asn	Ala	Ser	Ala	Ser	Gln	Ala	Ser	Cys	Ser	Ser	Asp
	415					420					425				
GGC	TAC	CCG	CTA	CCC	TCT	TGG	ACC	TGG	AAG	AAG	TGT	TCG	GAC	AAA	TCT
Gly	Tyr	Pro	Leu	Pro	Ser	Trp	Thr	Trp	Lys	Lys	Cys	Ser	Asp	Lys	Ser
430					435					440					445
CCC	AAT	TGC	ACG	GAG	GAA	ATC	CCA	GAA	GGA	GTT	TGG	AAT	AAA	AAG	GCT
Pro	Asn	Cys	Thr	Glu	Glu	Ile	Pro	Glu	Gly	Val	Trp	Asn	Lys	Lys	Ala
				450					455					460	
AAC	AGA	AAA	GTG	TTT	GGC	CAG	TGG	GTG	TCG	AGC	AGT	ACT	CTA	AAT	ATG
Asn	Arg	Lys	Val	Phe	Gly	Gln	Trp	Val	Ser	Ser	Ser	Thr	Leu	Asn	Met
			465					470					475		
AGT	GAG	GCC	GGG	AAA	GGG	CTT	CTG	GTC	AAA	TGC	TGT	GCG	TAC	AAT	TCT
Ser	Glu	Ala	Gly	Lys	Gly	Leu	Leu	Val	Lys	Cys	Cys	Ala	Tyr	Asn	Ser
		480					485					490			
ATG	GGC	ACG	TCT	TGC	GAA	ACC	ATC	TTT	TTA	AAC	TCA	CCA	GGC	CCC	TTC
Met	Gly	Thr	Ser	Cys	Glu	Thr	Ile	Phe	Leu	Asn	Ser	Pro	Gly	Pro	Phe
	495					500					505				
CCT	TTC	ATC	CAA	GAC	AAC	ATC	TCC	TTC	TAT	GCG	ACC	ATT	GGG	CTC	TGT
Pro	Phe	Ile	Gln	Asp	Asn	Ile	Ser	Phe	Tyr	Ala	Thr	Ile	Gly	Leu	Cys
510					515					520					525
CTC	CCC	TTC	ATT	GTT	GTT	CTC	ATT	GTG	TTG	ATC	TGC	CAC	AAA	TAC	AAA
Leu	Pro	Phe	Ile	Val	Val	Leu	Ile	Val	Leu	Ile	Cys	His	Lys	Tyr	Lys
				530					535					540	

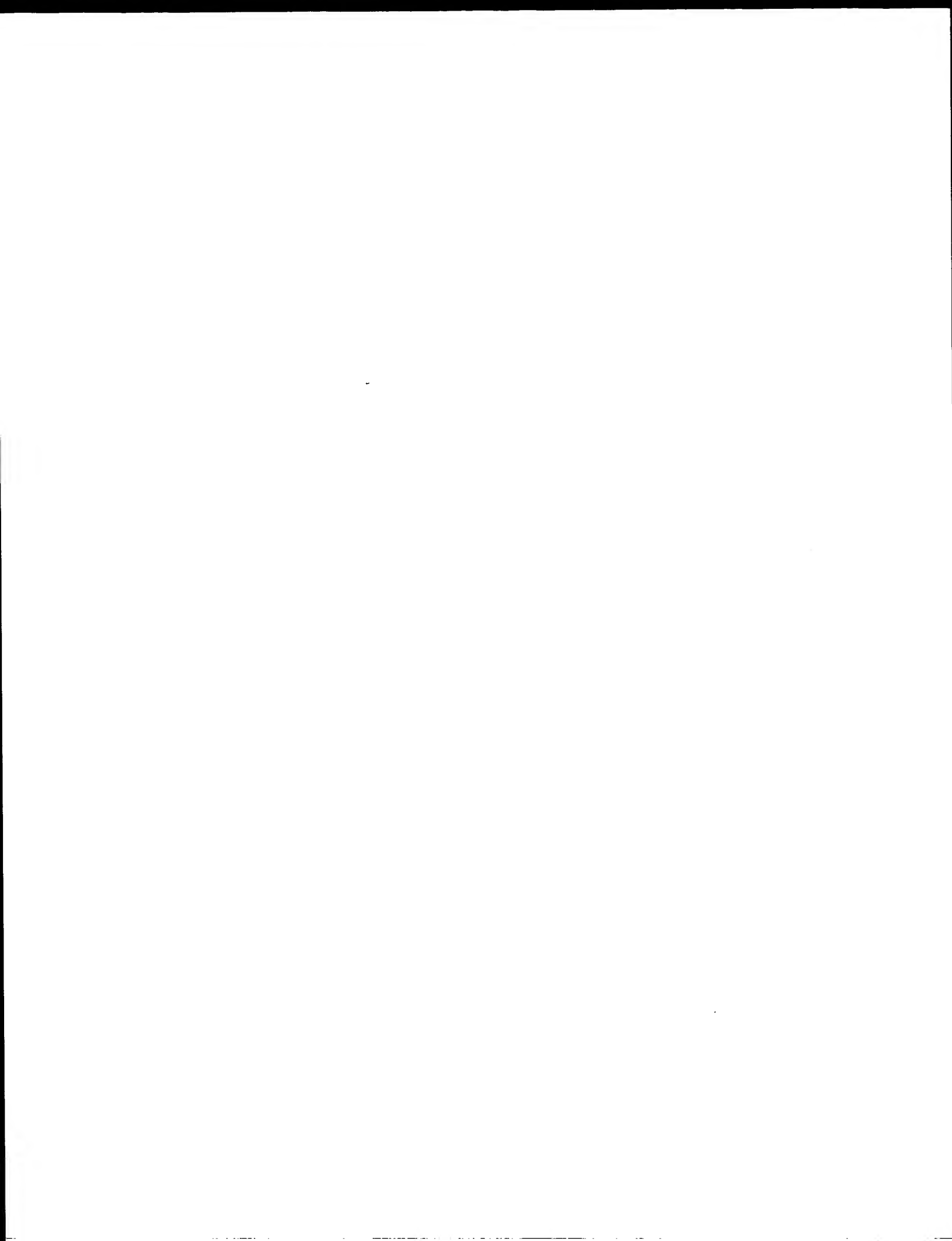


Fig. 1a.4

AAG	CAA	TTT	AGG	TAC	GAG	AGT	CAG	CTG	CAG	ATG	ATC	CAG	GTG	ACT	GGC			
Lys	Gln	Phe	Arg	Tyr	Glu	Ser	Gln	Leu	Gln	Met	Ile	Gln	Val	Thr	Gly			
			545															
CCC	CTG	GAT	AAC	GAG	TAC	TTC	TAC	GTT	GAC	TTC	AGG	GAC	TAT	GAA	TAT			
Pro	Leu	Asp	Asn	Glu	Tyr	Phe	Tyr	Val	Asp	Phe	Arg	Asp	Tyr	Glu	Tyr			
			560															
GAC	CTT	AAG	TGG	GAG	TTC	CCG	AGA	GAG	AAC	TTA	GAG	TTT	GGG	AAG	GTC			
Asp	Leu	Lys	Trp	Glu	Phe	Pro	Arg	Glu	Asn	Leu	Glu	Phe	Gly	Lys	Val			
			575															
CTG	GGG	TCT	GGC	GCT	TTC	GGG	AGG	GTG	ATG	AAC	GCC	ACG	GCC	TAT	GGC			
Leu	Gly	Ser	Gly	Ala	Phe	Gly	Arg	Val	Met	Asn	Ala	Thr	Ala	Tyr	Gly			
ATT	AGT	AAA	ACG	GGA	GTC	TCA	ATT	CAG	GTG	GCG	GTG	AAG	ATG	CTA	AAA			
Ile	Ser	Lys	Thr	Gly	Val	Ser	Ile	Gln	Val	Ala	Val	Lys	Met	Leu	Lys			
GAG	AAA	GCT	GAC	AGC	TGT	GAA	AAA	GAA	GCT	CTC	ATG	TCG	GAG	CTC	AAA			
Glu	Lys	Ala	Asp	Ser	Cys	Glu	Lys	Glu	Ala	Leu	Met	Ser	Glu	Leu	Lys			
ATG	ATG	ACC	CAC	CTG	GGA	CAC	CAT	GAC	AAC	ATC	GTG	AAT	CTG	CTG	GGG			
Met	Met	Thr	His	Leu	Gly	His	His	Asp	Asn	Ile	Val	Asn	Leu	Leu	Gly			
GCA	TGC	ACA	CTG	TCA	GGG	CCA	GTG	TAC	TTG	ATT	TTT	GAA	TAT	TGT	TGC			
Ala	Cys	Thr	Leu	Ser	Gly	Pro	Val	Tyr	Leu	Ile	Phe	Glu	Tyr	Cys	Cys			
TAT	GGT	GAC	CTC	CTC	AAC	TAC	CTA	AGA	AGT	AAA	AGA	GAG	AAG	TTT	CAC			
Tyr	Gly	Asp	Leu	Leu	Asn	Tyr	Leu	Arg	Ser	Lys	Arg	Glu	Lys	Phe	His			
AGG	ACA	TGG	ACA	GAG	ATT	TTT	AAG	GAA	CAT	AAT	TTC	AGT	TCT	TAC	CCT			
Arg	Thr	Trp	Thr	Glu	Ile	Phe	Lys	Glu	His	Asn	Phe	Ser	Ser	Tyr	Pro			
ACT	TTC	CAG	GCA	CAT	TCA	AAT	TCC	AGC	ATG	CCT	GGT	TCA	CGA	GAA	GTT			
Thr	Phe	Gln	Ala	His	Ser	Asn	Ser	Ser	Met	Pro	Gly	Ser	Arg	Glu	Val			
CAG	TTA	CAC	CCG	CCC	TTG	GAT	CAG	CTC	TCA	GGG	TTC	AAT	GGG	AAT	TCA			
Gln	Leu	His	Pro	Pro	Leu	Asp	Gln	Leu	Ser	Gly	Phe	Asn	Gly	Asn	Ser			

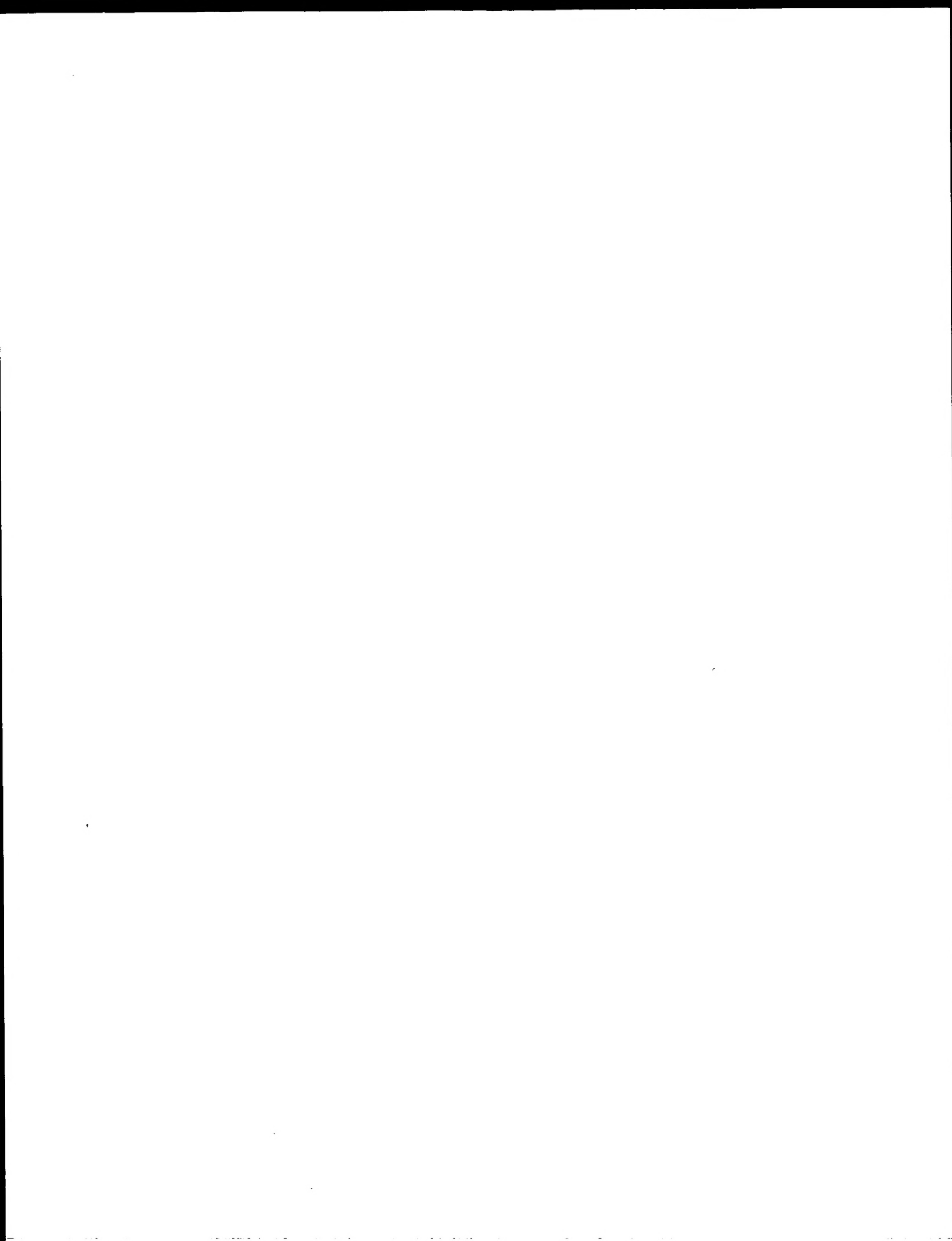
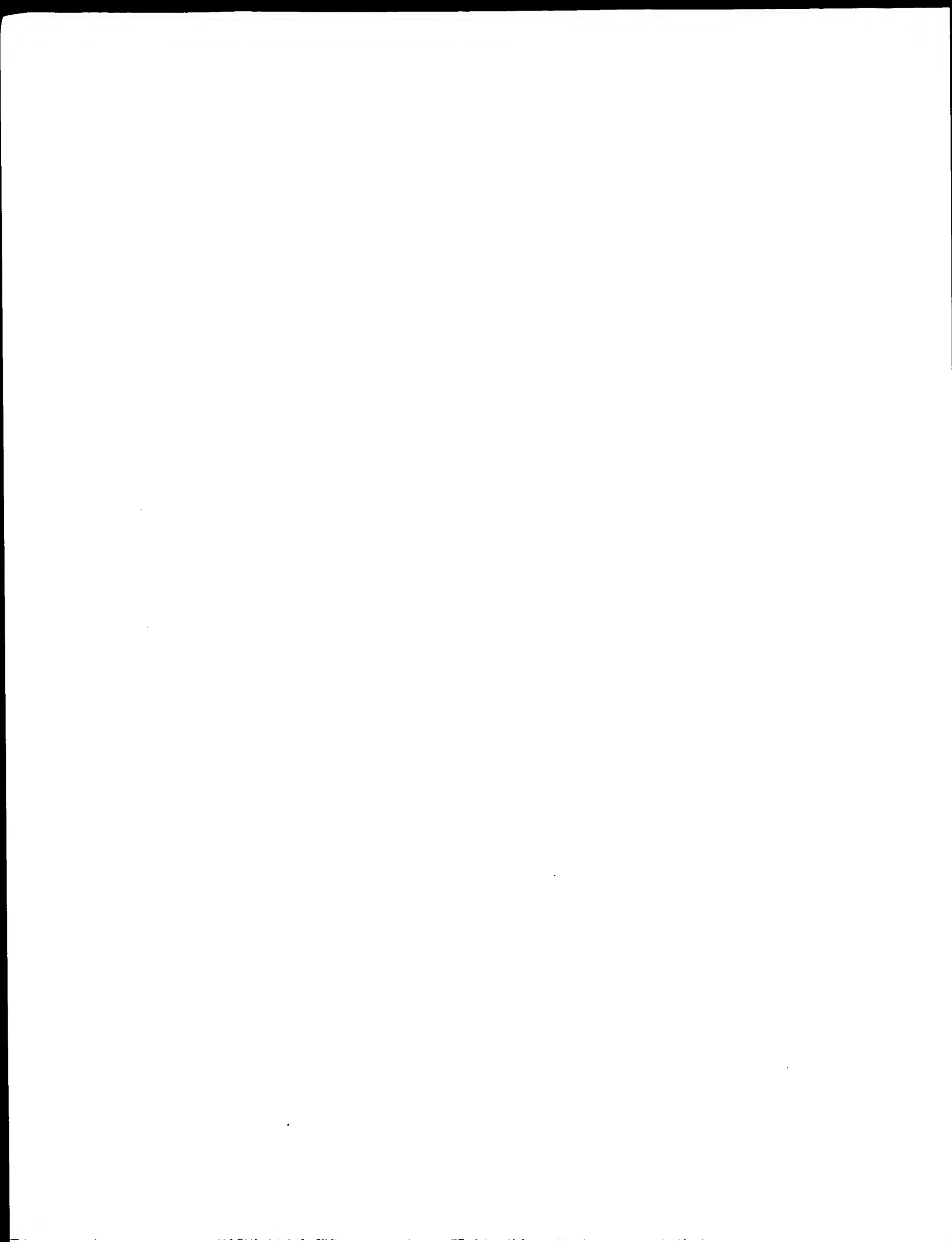


Fig. 1a.5

ATT Ile	CAT His	TCT Ser	GAA Glu	GAT Asp	GAG Glu	ATT Ile	GAA Glu	TAT Tyr	GAA Glu	AAC Asn	CAG Gln	AAG Lys	AGG Arg	CTG Leu	GCA Ala	
	735					740					745					
GAA Glu	GAA Glu	GAG Glu	GAG Glu	GAA Glu	GAT Asp	TTG Leu	AAC Asn	GTG Val	CTG Leu	ACG Thr	TTT Phe	GAA Glu	GAC Asp	CTC Leu	CTT Leu	
	750				755					760					765	
TGC Cys	TTT Phe	GCG Ala	TAC Tyr	CAA Gln	GTG Val	GCC Ala	AAA Lys	GGC Gly	ATG Met	GAA Glu	TTC Phe	CTG Leu	GAG Glu	TTC Phe	AAG Lys	
				770					775						780	
TCG Ser	TGT Cys	GTC Val	CAC His	AGA Arg	GAC Asp	CTG Leu	GCA Ala	GCC Ala	AGG Arg	AAT Asn	GTG Val	TTG Leu	GTC Val	ACC Thr	CAC His	
			785					790						795		
GGG Gly	AAG Lys	GTG Val	GTG Val	AAG Lys	ATC Ile	TGT Cys	GAC Asp	TTT Phe	GGA Gly	CTG Leu	GCC Ala	CGA Arg	GAC Asp	ATC Ile	CTG Leu	
		800					805					810				
AGC Ser	GAC Asp	TCC Ser	AGC Ser	TAC Tyr	GTC Val	GTC Val	AGG Arg	GGC Gly	AAC Asn	GCA Ala	CGG Arg	CTG Leu	CCG Pro	GTG Val	AAG Lys	
	815					820					825					
TGG Trp	ATG Met	GCA Ala	CCC Pro	GAG Glu	AGC Ser	TTA Leu	TTT Phe	GAA Glu	GGG Gly	ATC Ile	TAC Tyr	ACA Thr	ATC Ile	AAG Lys	AGT Ser	
	830				835					840					845	
GAC Asp	GTC Val	TGG Trp	TCC Ser	TAC Tyr	GGC Gly	ATC Ile	CTT Leu	CTC Leu	TGG Trp	GAG Glu	ATA Ile	TTT Phe	TCA Ser	CTG Leu	GGT Gly	
				850					855					860		
GTG Val	AAC Asn	CCT Pro	TAC Tyr	CCT Pro	GGC Gly	ATT Ile	CCT Pro	GTC Val	GAC Asp	GCT Ala	AAC Asn	TTC Phe	TAT Tyr	AAA Lys	CTG Leu	
			865					870					875			
ATT Ile	CAG Gln	AGT Ser	GGA Gly	TTT Phe	AAA Lys	ATG Met	GAG Glu	CAG Gln	CCA Pro	TTC Phe	TAT Tyr	GCC Ala	ACA Thr	GAA Glu	GGG Gly	
		880					885					890				
ATA Ile	TAC Tyr	TTT Phe	GTA Val	ATG Met	CAA Gln	TCC Ser	TGC Cys	TGG Trp	GCT Ala	TTT Phe	GAC Asp	TCA Ser	AGG Arg	AAG Lys	CGG Arg	
	895					900					905					



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Fig. 1a.6

CCA	TCC	TTC	CCC	AAC	CTG	ACT	TCA	TTT	TTA	GGA	TGT	CAG	CTG	GCA	GAG
Pro	Ser	Phe	Pro	Asn	Leu	Thr	Ser	Phe	Leu	Gly	Cys	Gln	Leu	Ala	Glu
910					915					920					925

GCA	GAA	GAA	GCA	TGT	ATC	AGA	ACA	TCC	ATC	CAT	CTA	CCA	AAA	CAG	GCG
Ala	Glu	Glu	Ala	Cys	Ile	Arg	Thr	Ser	Ile	His	Leu	Pro	Lys	Gln	Ala
				930					935					940	

GCC	CCT	CAG	CAG	AGA	GGC	GGG	CTC	AGA	GCC	CAG	TCG	CCA	CAG	CGC	CAG
Ala	Pro	Gln	Gln	Arg	Gly	Gly	Leu	Arg	Ala	Gln	Ser	Pro	Gln	Arg	Gln
			945					950					955		

GTG	AAG	ATT	CAC	AGA	GAA	AGA	AGT	TAGCGAGGAG	GCCTTGGACC	CCGCCACCCT
Val	Lys	Ile	His	Arg	Glu	Arg	Ser			
		960					965			

AGCAGGCTGT AGACCGCAGA GCCAAGATTA GCCTCGCCTC TGAGGAAGCG CCCTACAGCG

CGTTGCTTCG CTGGACTTTT CTCTAGATGC TGTCTGCCAT TACTCCAAAG TGACTTCTAT

AAAATCAAAC CTCTCCTCGC ACAGGCGGGA GAGCCAATAA TGAGACTTGT TGGTGAGCCC

GCCTACCCTG GGGGCCTTTC CACGAGCTTG AGGGGAAAGC CATGTATCTG AAATATAGTA

TATTCTTGTA AATACGTGAA ACAAACCAA CCCGTTTTTTT GCTAAGGGAA AGCTAAATAT

GATTTTTTAAA AATCTATGTT TTAAATACT ATGTAACCTT TTCATCTATT TAGTGATATA

TTTTATGGAT GGAAATAAAC TTTCTACTGT AAAAAAAAAA AAAAAAAAAA AAAAAA

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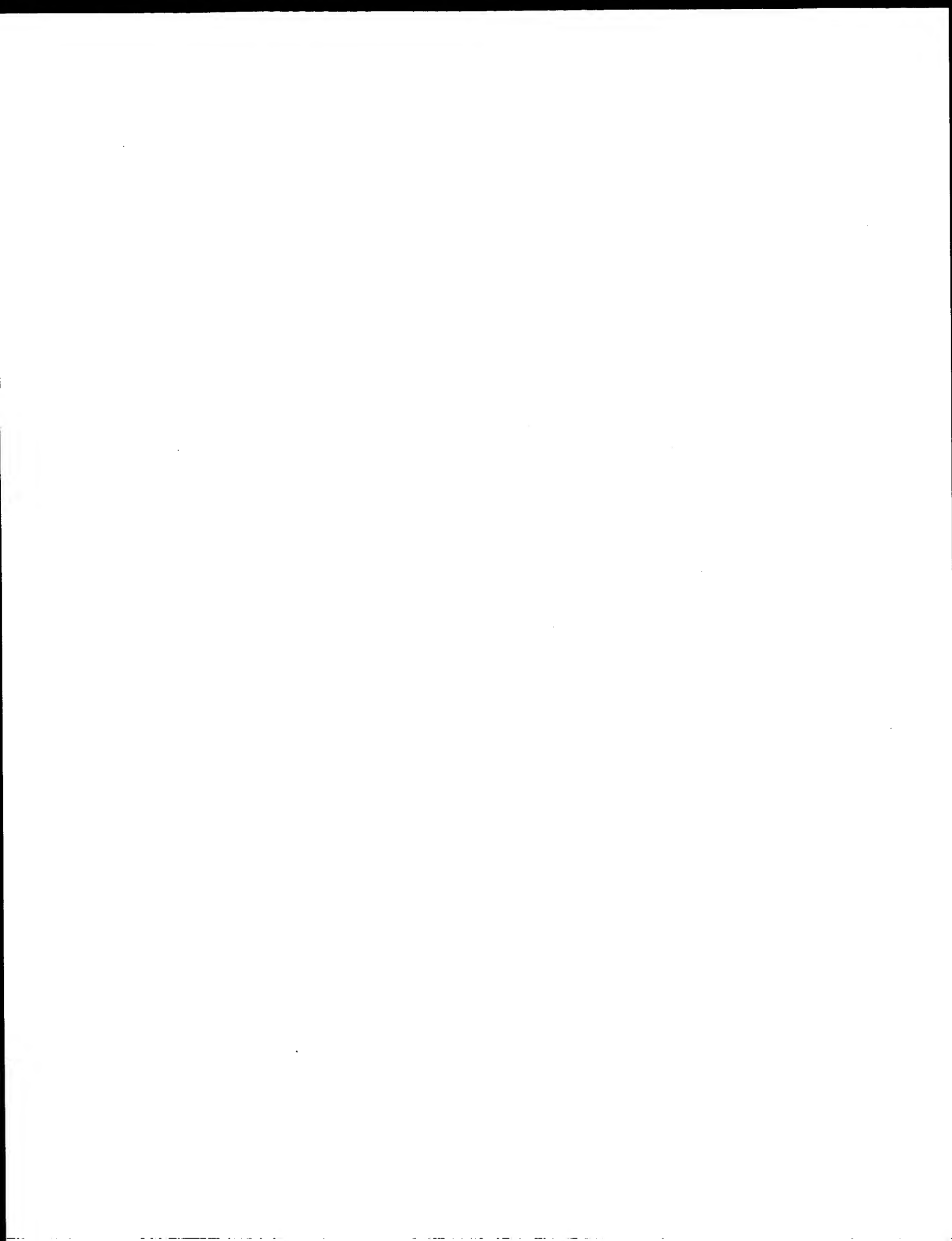


Fig. 1b.1

CGAGGCGGCA TCCGAGGGCT GGGCCGGCGC CCTGGGGGAC CCCGGGCTCC GGAGGCC

ATG CCG GCG TTG GCG CGC GAC GCG GGC ACC GTG CCG CTG CTC GTT GTT
Met Pro Ala Leu Ala Arg Asp Ala Gly Thr Val Pro Leu Leu Val Val
-27 -25 -20 -15

TTT TCT GCA ATG ATA TTT GGG ACT ATT ACA AAT CAA GAT CTG CCT GTG
Phe Ser Ala Met Ile Phe Gly Thr Ile Thr Asn Gln Asp Leu Pro Val
-10 -5 1 5

ATC AAG TGT GTT TTA ATC AAT CAT AAG AAC AAT GAT TCA TCA GTG GGG
Ile Lys Cys Val Leu Ile Asn His Lys Asn Asn Asp Ser Ser Val Gly
10 15 20

AAG TCA TCA TCA TAT CCC ATG GTA TCA GAA TCC CCG GAA GAC CTC GGG
Lys Ser Ser Ser Tyr Pro Met Val Ser Glu Ser Pro Glu Asp Leu Gly
25 30 35

TGT GCG TTG AGA CCC CAG AGC TCA GGG ACA GTG TAC GAA GCT GCC GCT
Cys Ala Leu Arg Pro Gln Ser Ser Gly Thr Val Tyr Glu Ala Ala Ala
40 45 50

GTG GAA GTG GAT GTA TCT GCT TCC ATC ACA CTG CAA GTG CTG GTC GAT
Val Glu Val Asp Val Ser Ala Ser Ile Thr Leu Gln Val Leu Val Asp
55 60 65

GCC CCA GGG AAC ATT TCC TGT CTC TGG GTC TTT AAG CAC AGC TCC CTG
Ala Pro Gly Asn Ile Ser Cys Leu Trp Val Phe Lys His Ser Ser Leu
70 75 80 85

AAT TGC CAG CCA CAT TTT GAT TTA CAA AAC AGA GGA GTT GTT TCC ATG
Asn Cys Gln Pro His Phe Asp Leu Gln Asn Arg Gly Val Val Ser Met
90 95 100

GTC ATT TTG AAA ATG ACA GAA ACC CAA GCT GGA GAA TAC CTA CTT TTT
Val Ile Leu Lys Met Thr Glu Thr Gln Ala Gly Glu Tyr Leu Leu Phe
105 110 115

ATT CAG AGT GAA GCT ACC AAT TAC ACA ATA TTG TTT ACA GTG AGT ATA
Ile Gln Ser Glu Ala Thr Asn Tyr Thr Ile Leu Phe Thr Val Ser Ile
120 125 130

AGA AAT ACC CTG CTT TAC ACA TTA AGA AGA CCT TAC TTT AGA AAA ATG
Arg Asn Thr Leu Leu Tyr Thr Leu Arg Arg Pro Tyr Phe Arg Lys Met
135 140 145

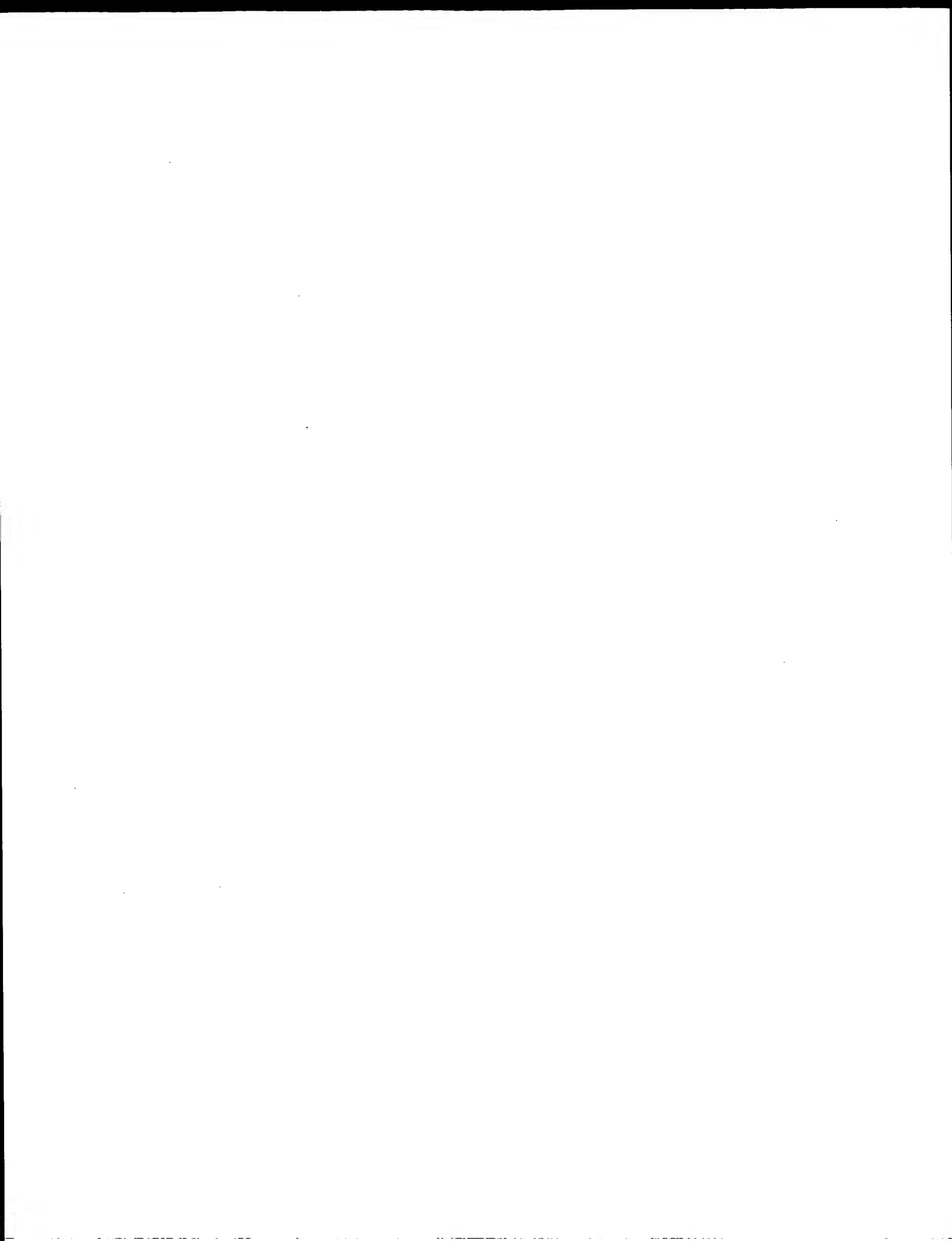


Fig. 1b.2

GAA Glu 150	AAC Asn	CAG Gln	GAC Asp	GCC Ala	CTG Leu 155	GTC Val	TGC Cys	ATA Ile	TCT Ser	GAG Glu 160	AGC Ser	GTT Val	CCA Pro	GAG Glu	CCG Pro 165
ATC Ile	GTG Val	GAA Glu	TGG Trp	GTG Val 170	CTT Leu	TGC Cys	GAT Asp	TCA Ser	CAG Gln 175	GGG Gly	GAA Glu	AGC Ser	TGT Cys	AAA Lys 180	GAA Glu
GAA Glu	AGT Ser	CCA Pro	GCT Ala 185	GTT Val	GTT Val	AAA Lys	AAG Lys	GAG Glu 190	GAA Glu	AAA Lys	GTG Val	CTT Leu	CAT His 195	GAA Glu	TTA Leu
TTT Phe	GGG Gly	ACG Thr 200	GAC Asp	ATA Ile	AGG Arg	TGC Cys	TGT Cys 205	GCC Ala	AGA Arg	AAT Asn	GAA Glu	CTG Leu 210	GGC Gly	AGG Arg	GAA Glu
TGC Cys 215	ACC Thr	AGG Arg	CTG Leu	TTC Phe	ACA Thr 220	ATA Ile	GAT Asp	CTA Leu	AAT Asn	CAA Gln	ACT Thr 225	CCT Pro	CAG Gln	ACC Thr	ACA Thr
TTG Leu 230	CCA Pro	CAA Gln	TTA Leu	TTT Phe	CTT Leu 235	AAA Lys	GTA Val	GGG Gly	GAA Glu	CCC Pro 240	TTA Leu	TGG Trp	ATA Ile	AGG Arg	TGC Cys 245
AAA Lys	GCT Ala	GTT Val	CAT His 250	GTG Val	AAC Asn	CAT His	GGA Gly	TTC Phe	GGG Gly 255	CTC Leu	ACC Thr	TGG Trp	GAA Glu	TTA Leu 260	GAA Glu
AAC Asn	AAA Lys	GCA Ala	CTC Leu 265	GAG Glu	GAG Glu	GGC Gly	AAC Asn	TAC Tyr 270	TTT Phe	GAG Glu	ATG Met	AGT Ser	ACC Thr 275	TAT Tyr	TCA Ser
ACA Thr	AAC Asn	AGA Arg 280	ACT Thr	ATG Met	ATA Ile	CGG Arg	ATT Ile 285	CTG Leu	TTT Phe	GCT Ala	TTT Phe 290	GTA Val	TCA Ser	TCA Ser	GTG Val
GCA Ala 295	AGA Arg	AAC Asn	GAC Asp	ACC Thr	GGA Gly	TAC Tyr 300	TAC Tyr	ACT Thr	TGT Cys	TCC Ser 305	TCT Ser	TCA Ser	AAG Lys	CAT His	CCC Pro
AGT Ser 310	CAA Gln	TCA Ser	GCT Ala	TTG Leu	GTT Val 315	ACC Thr	ATC Ile	GTA Val	GGA Gly	AAG Lys 320	GGA Gly	TTT Phe	ATA Ile	AAT Asn	GCT Ala 325
ACC Thr	AAT Asn	TCA Ser	AGT Ser	GAA Glu 330	GAT Asp	TAT Tyr	GAA Glu	ATT Ile	GAC Asp 335	CAA Gln	TAT Tyr	GAA Glu	GAG Glu	TTT Phe 340	TGT Cys

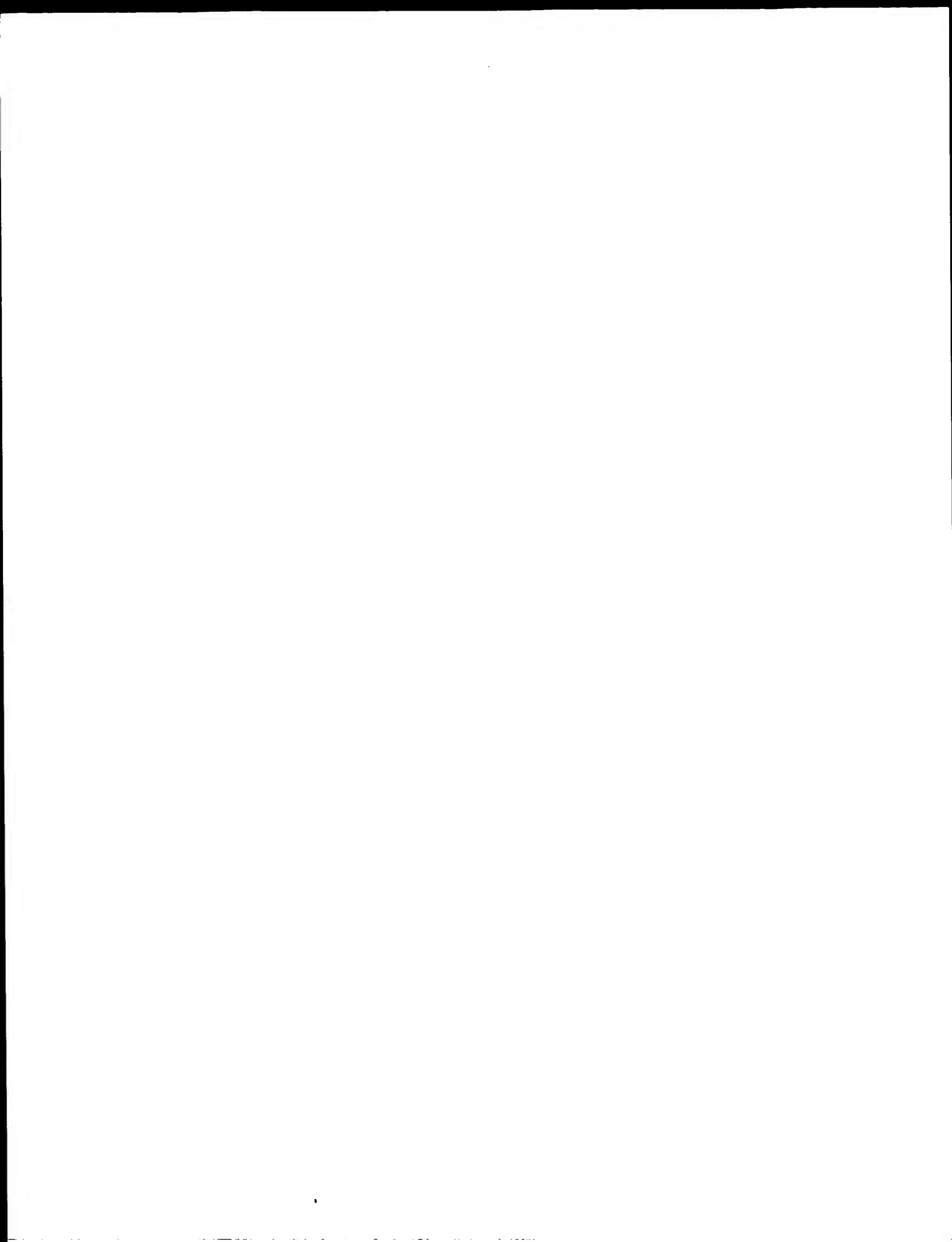


Fig. 1b.3

TTT	TCT	GTC	AGG	TTT	AAA	GCC	TAC	CCA	CAA	ATC	AGA	TGT	ACG	TGG	ACC			
Phe	Ser	Val	Arg	Phe	Lys	Ala	Tyr	Pro	Gln	Ile	Arg	Cys	Thr	Trp	Thr			
			345					350					355					
TTC	TCT	CGA	AAA	TCA	TTT	CCT	TGT	GAG	CAA	AAG	GGT	CTT	GAT	AAC	GGA			
Phe	Ser	Arg	Lys	Ser	Phe	Pro	Cys	Glu	Gln	Lys	Gly	Leu	Asp	Asn	Gly			
		360					365					370						
TAC	AGC	ATA	TCC	AAG	TTT	TGC	AAT	CAT	AAG	CAC	CAG	CCA	GGA	GAA	TAT			
Tyr	Ser	Ile	Ser	Lys	Phe	Cys	Asn	His	Lys	His	Gln	Pro	Gly	Glu	Tyr			
	375					380					385							
ATA	TTC	CAT	GCA	GAA	AAT	GAT	GAT	GCC	CAA	TTT	ACC	AAA	ATG	TTC	ACG			
Ile	Phe	His	Ala	Glu	Asn	Asp	Asp	Ala	Gln	Phe	Thr	Lys	Met	Phe	Thr			
390					395					400					405			
CTG	AAT	ATA	AGA	AGG	AAA	CCT	CAA	GTG	CTC	GCA	GAA	GCA	TCG	GCA	AGT			
Leu	Asn	Ile	Arg	Arg	Lys	Pro	Gln	Val	Leu	Ala	Glu	Ala	Ser	Ala	Ser			
				410					415					420				
CAG	GCG	TCC	TGT	TTC	TCG	GAT	GGA	TAC	CCA	TTA	CCA	TCT	TGG	ACC	TGG			
Gln	Ala	Ser	Cys	Phe	Ser	Asp	Gly	Tyr	Pro	Leu	Pro	Ser	Trp	Thr	Trp			
			425					430					435					
AAG	AAG	TGT	TCA	GAC	AAG	TCT	CCC	AAC	TGC	ACA	GAA	GAG	ATC	ACA	GAA			
Lys	Lys	Cys	Ser	Asp	Lys	Ser	Pro	Asn	Cys	Thr	Glu	Glu	Ile	Thr	Glu			
		440					445					450						
GGA	GTC	TGG	AAT	AGA	AAG	GCT	AAC	AGA	AAA	GTG	TTT	GGA	CAG	TGG	GTG			
Gly	Val	Trp	Asn	Arg	Lys	Ala	Asn	Arg	Lys	Val	Phe	Gly	Gln	Trp	Val			
	455					460					465							
TCG	AGC	AGT	ACT	CTA	AAC	ATG	AGT	GAA	GCC	ATA	AAA	GGG	TTC	CTG	GTC			
Ser	Ser	Ser	Thr	Leu	Asn	Met	Ser	Glu	Ala	Ile	Lys	Gly	Phe	Leu	Val			
470					475					480					485			
AAG	TGC	TGT	GCA	TAC	AAT	TCC	CTT	GGC	ACA	TCT	TGT	GAG	ACG	ATC	CTT			
Lys	Cys	Cys	Ala	Tyr	Asn	Ser	Leu	Gly	Thr	Ser	Cys	Glu	Thr	Ile	Leu			
				490					495					500				
TTA	AAC	TCT	CCA	GGC	CCC	TTC	CCT	TTC	ATC	CAA	GAC	AAC	ATC	TCA	TTC			
Leu	Asn	Ser	Pro	Gly	Pro	Phe	Pro	Phe	Ile	Gln	Asp	Asn	Ile	Ser	Phe			
			505					510					515					
TAT	GCA	ACA	ATT	GGT	GTT	TGT	CTC	CTC	TTC	ATT	GTC	GTT	TTA	ACC	CTG			
Tyr	Ala	Thr	Ile	Gly	Val	Cys	Leu	Leu	Phe	Ile	Val	Val	Leu	Thr	Leu			
		520					525					530						

Fig. 1b.4

CTA Leu 535	ATT Ile	TGT Cys	CAC His	AAG Lys	TAC Tyr	AAA Lys 540	AAG Lys	CAA Gln	TTT Phe	AGG Arg	TAT Tyr 545	GAA Glu	AGC Ser	CAG Gln	CTA Leu
CAG Gln 550	ATG Met	GTA Val	CAG Gln	GTG Val	ACC Thr 555	GGC Gly	TCC Ser	TCA Ser	GAT Asp	AAT Asn 560	GAG Glu	TAC Tyr	TTC Phe	TAC Tyr	GTT Val 565
GAT Asp	TTC Phe	AGA Arg	GAA Glu	TAT Tyr 570	GAA Glu	TAT Tyr	GAT Asp	CTC Leu	AAA Lys 575	TGG Trp	GAG Glu	TTT Phe	CCA Pro	AGA Arg 580	GAA Glu
AAT Asn	TTA Leu	GAG Glu	TTT Phe 585	GGG Gly	AAG Lys	GTA Val	CTA Leu	GGA Gly 590	TCA Ser	GGT Gly	GCT Ala	TTT Phe	GGA Gly 595	AAA Lys	GTG Val
ATG Met	AAC Asn	GCA Ala 600	ACA Thr	GCT Ala	TAT Tyr	GGA Gly	ATT Ile 605	AGC Ser	AAA Lys	ACA Thr	GGA Gly	GTC Val 610	TCA Ser	ATC Ile	CAG Gln
GTT Val 615	GCC Ala	GTC Val	AAA Lys	ATG Met	CTG Leu	AAA Lys 620	GAA Glu	AAA Lys	GCA Ala	GAC Asp	AGC Ser 625	TCT Ser	GAA Glu	AGA Arg	GAG Glu
GCA Ala 630	CTC Leu	ATG Met	TCA Ser	GAA Glu	CTC Leu 635	AAG Lys	ATG Met	ATG Met	ACC Thr	CAG Gln 640	CTG Leu	GGA Gly	AGC Ser	CAC His	GAG Glu 645
AAT Asn	ATT Ile	GTG Val	AAC Asn	CTG Leu 650	CTG Leu	GGG Gly	GCG Ala	TGC Cys	ACA Thr 655	CTG Leu	TCA Ser	GGA Gly	CCA Pro	ATT Ile 660	TAC Tyr
TTG Leu	ATT Ile	TTT Phe	GAA Glu 665	TAC Tyr	TGT Cys	TGC Cys	TAT Tyr	GGT Gly 670	GAT Asp	CTT Leu	CTC Leu	AAC Asn	TAT Tyr 675	CTA Leu	AGA Arg
AGT Ser	AAA Lys	AGA Arg 680	GAA Glu	AAA Lys	TTT Phe	CAC His	AGG Arg 685	ACT Thr	TGG Trp	ACA Thr	GAG Glu	ATT Ile 690	TTC Phe	AAG Lys	GAA Glu
CAC His 695	AAT Asn	TTC Phe	AGT Ser	TTT Phe	TAC Tyr	CCC Pro 700	ACT Thr	TTC Phe	CAA Gln	TCA Ser	CAT His 705	CCA Pro	AAT Asn	TCC Ser	AGC Ser
ATG Met 710	CCT Pro	GGT Gly	TCA Ser	AGA Arg	GAA Glu 715	GTT Val	CAG Gln	ATA Ile	CAC His	CCG Pro 720	GAC Asp	TCG Ser	GAT Asp	CAA Gln	ATC Ile 725

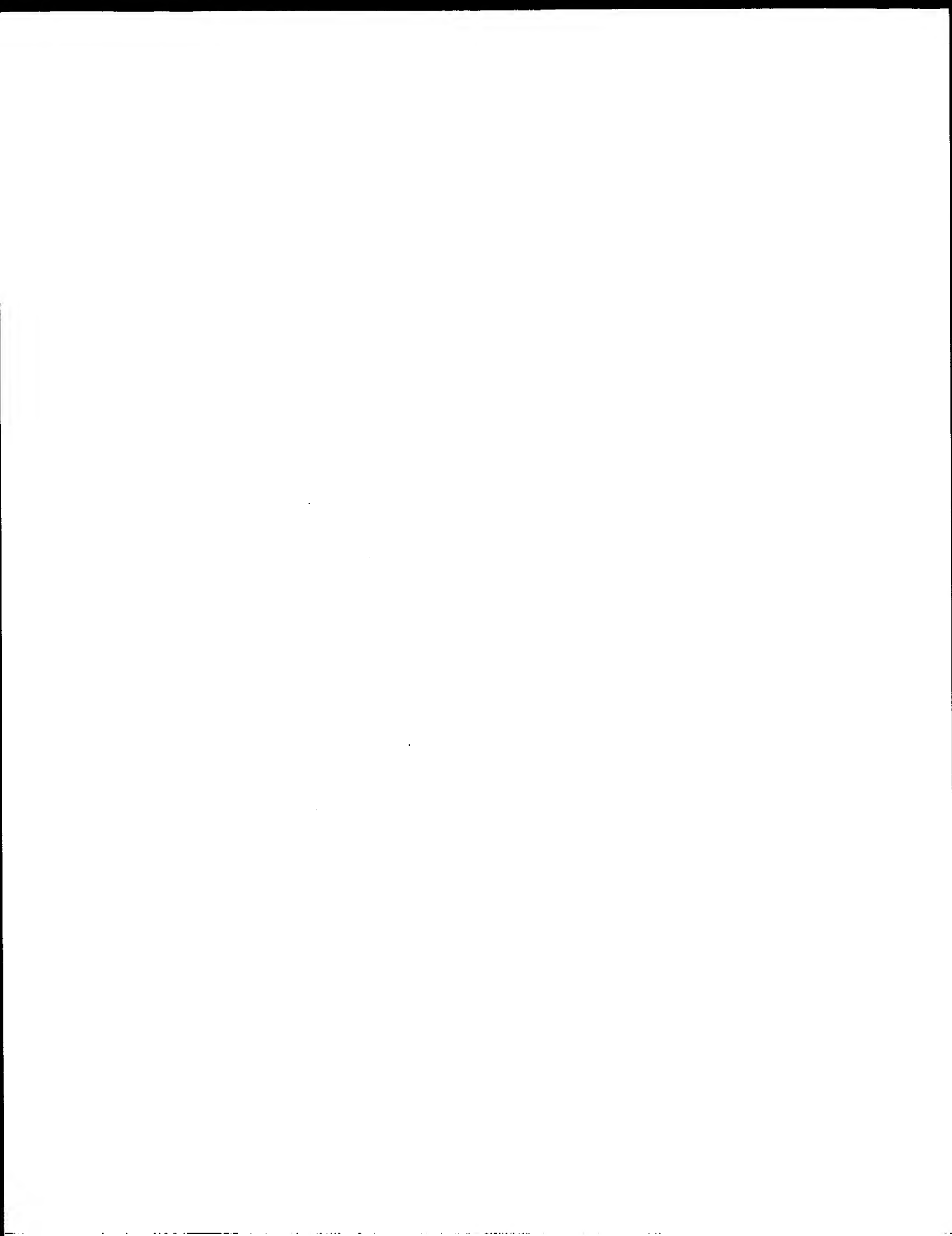


Fig. 1b.5

TCA	GGG	CTT	CAT	GGG	AAT	TCA	TTT	CAC	TCT	GAA	GAT	GAA	ATT	GAA	TAT	
Ser	Gly	Leu	His	Gly	Asn	Ser	Phe	His	Ser	Glu	Asp	Glu	Ile	Glu	Tyr	
				730					735					740		
GAA	AAC	CAA	AAA	AGG	CTG	GAA	GAA	GAG	GAG	GAC	TTG	AAT	GTG	CTT	ACA	
Glu	Asn	Gln	Lys	Arg	Leu	Glu	Glu	Glu	Glu	Asp	Leu	Asn	Val	Leu	Thr	
			745					750					755			
TTT	GAA	GAT	CTT	CTT	TGC	TTT	GCA	TAT	CAA	GTT	GCC	AAA	GGA	ATG	GAA	
Phe	Glu	Asp	Leu	Leu	Cys	Phe	Ala	Tyr	Gln	Val	Ala	Lys	Gly	Met	Glu	
		760					765					770				
TTT	CTG	GAA	TTT	AAG	TCG	TGT	GTT	CAC	AGA	GAC	CTG	GCC	GCC	AGG	AAC	
Phe	Leu	Glu	Phe	Lys	Ser	Cys	Val	His	Arg	Asp	Leu	Ala	Ala	Arg	Asn	
	775				780						785					
GTG	CTT	GTC	ACC	CAC	GGG	AAA	GTG	GTG	AAG	ATA	TGT	GAC	TTT	GGA	TTG	
Val	Leu	Val	Thr	His	Gly	Lys	Val	Val	Lys	Ile	Cys	Asp	Phe	Gly	Leu	
	790				795					800					805	
GCT	CGA	GAT	ATC	ATG	AGT	GAT	TCC	AAC	TAT	GTT	GTC	AGG	GGC	AAT	GCC	
Ala	Arg	Asp	Ile	Met	Ser	Asp	Ser	Asn	Tyr	Val	Val	Arg	Gly	Asn	Ala	
				810					815					820		
CGT	CTG	CCT	GTA	AAA	TGG	ATG	GCC	CCC	GAA	AGC	CTG	TTT	GAA	GGC	ATC	
Arg	Leu	Pro	Val	Lys	Trp	Met	Ala	Pro	Glu	Ser	Leu	Phe	Glu	Gly	Ile	
			825					830					835			
TAC	ACC	ATT	AAG	AGT	GAT	GTC	TGG	TCA	TAT	GGA	ATA	TTA	CTG	TGG	GAA	
Tyr	Thr	Ile	Lys	Ser	Asp	Val	Trp	Ser	Tyr	Gly	Ile	Leu	Leu	Trp	Glu	
		840					845					850				
ATC	TTC	TCA	CTT	GGT	GTG	AAT	CCT	TAC	CCT	GGC	ATT	CCG	GTT	GAT	GCT	
Ile	Phe	Ser	Leu	Gly	Val	Asn	Pro	Tyr	Pro	Gly	Ile	Pro	Val	Asp	Ala	
	855					860					865					
AAC	TTC	TAC	AAA	CTG	ATT	CAA	AAT	GGA	TTT	AAA	ATG	GAT	CAG	CCA	TTT	
Asn	Phe	Tyr	Lys	Leu	Ile	Gln	Asn	Gly	Phe	Lys	Met	Asp	Gln	Pro	Phe	
	870				875					880					885	
TAT	GCT	ACA	GAA	GAA	ATA	TAC	ATT	ATA	ATG	CAA	TCC	TGC	TGG	GCT	TTT	
Tyr	Ala	Thr	Glu	Glu	Ile	Tyr	Ile	Ile	Met	Gln	Ser	Cys	Trp	Ala	Phe	
			890						895					900		
GAC	TCA	AGG	AAA	CGG	CCA	TCC	TTC	CCT	AAT	TTG	ACT	TCG	TTT	TTA	GGA	
Asp	Ser	Arg	Lys	Arg	Pro	Ser	Phe	Pro	Asn	Leu	Thr	Ser	Phe	Leu	Gly	
			905					910					915			

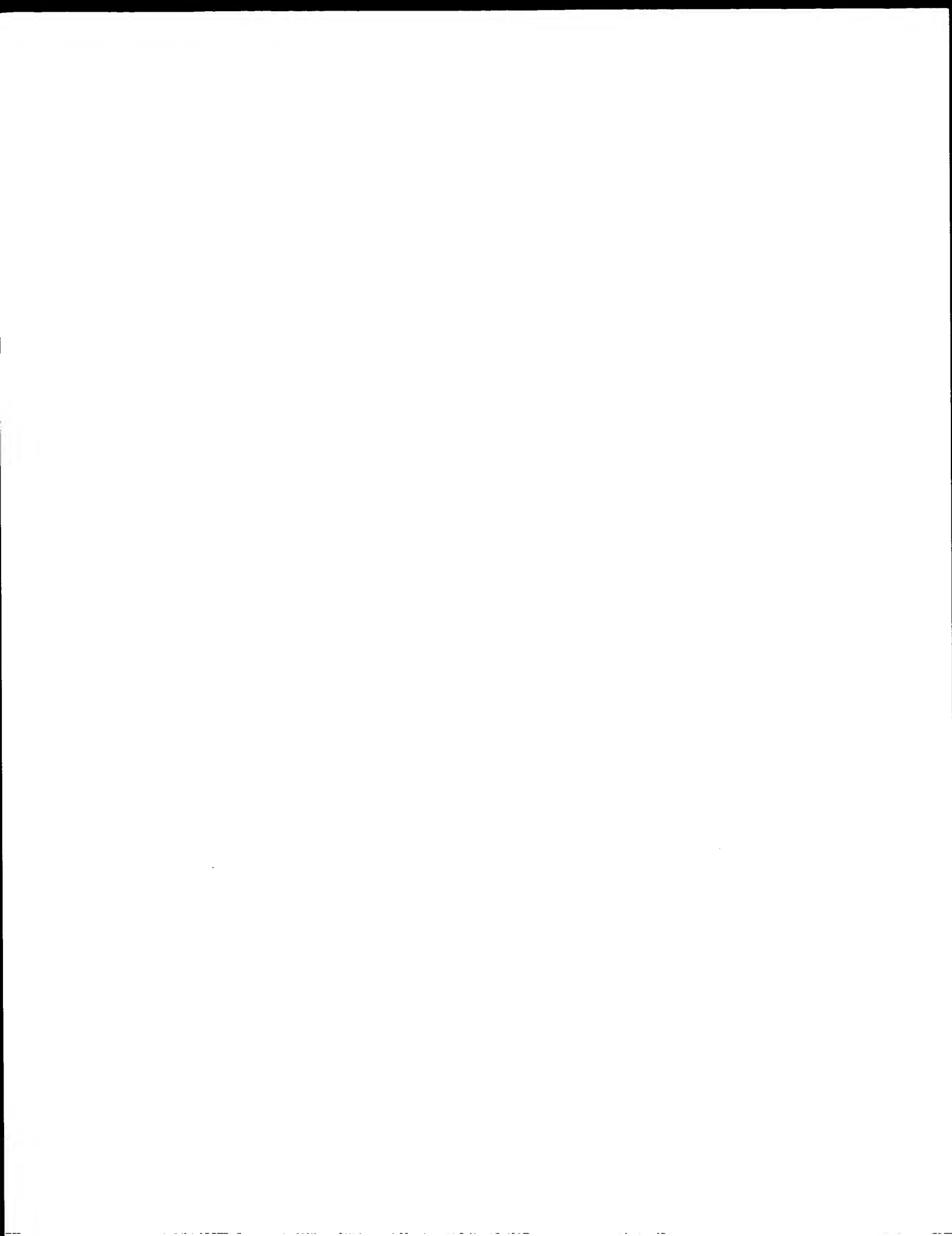


Fig. 1b.6

TGT	CAG	CTG	GCA	GAT	GCA	GAA	GAA	GCG	ATG	TAT	CAG	AAT	GTG	GAT	GGC
Cys	Gln	Leu	Ala	Asp	Ala	Glu	Glu	Ala	Met	Tyr	Gln	Asn	Val	Asp	Gly
		920						925					930		

CGT	GTT	TCG	GAA	TGT	CCT	CAC	ACC	TAC	CAA	AAC	AGG	CGA	CCT	TTC	AGC
Arg	Val	Ser	Glu	Cys	Pro	His	Thr	Tyr	Gln	Asn	Arg	Arg	Pro	Phe	Ser
	935					940					945				

AGA	GAG	ATG	GAT	TTG	GGG	CTA	CTC	TCT	CCG	CAG	GCT	CAG	GTC	GAA	GAT
Arg	Glu	Met	Asp	Leu	Gly	Leu	Leu	Ser	Pro	Gln	Ala	Gln	Val	Glu	Asp
950					955					960					965

TCG TAGAGGAACA ATTTAGTTTT AAGGACTTCA TCCCTCCACC TATCCCTAAC
Ser

AGGCTGTAGA TTACCAAAC AAGATTAATT TCATCACTAA AAGAAAATCT ATTATCAACT

GCTGCTTCAC CAGACTTTTC TCTAGAAGCC GTCTGCGTTT ACTCTTGTTT TCAAAGGGAC

TTTTGTAAAA TCAAATCATC CTGTCACAAG GCAGGAGGAG CTGATAATGA ACTTTATTGG

AGCATTGATC TGCATCCAAG GCCTTCTCAG GCCGGCTTGA GTGAATTGTG TACCTGAAGT

ACAGTATATT CTTGTAAATA CATAAAACAA AAGCATTTTG CTAAGGAGAA GCTAATATGA

TTTTTTAAGT CTATGTTTTA AAATAATATG TAAATTTTTC AGCTATTTAG TGATATATTT

TATGGGTGGG AATAAAATTT CTACTACAGA AAAAAAAAAA AAAAAAAAAA AAAAA

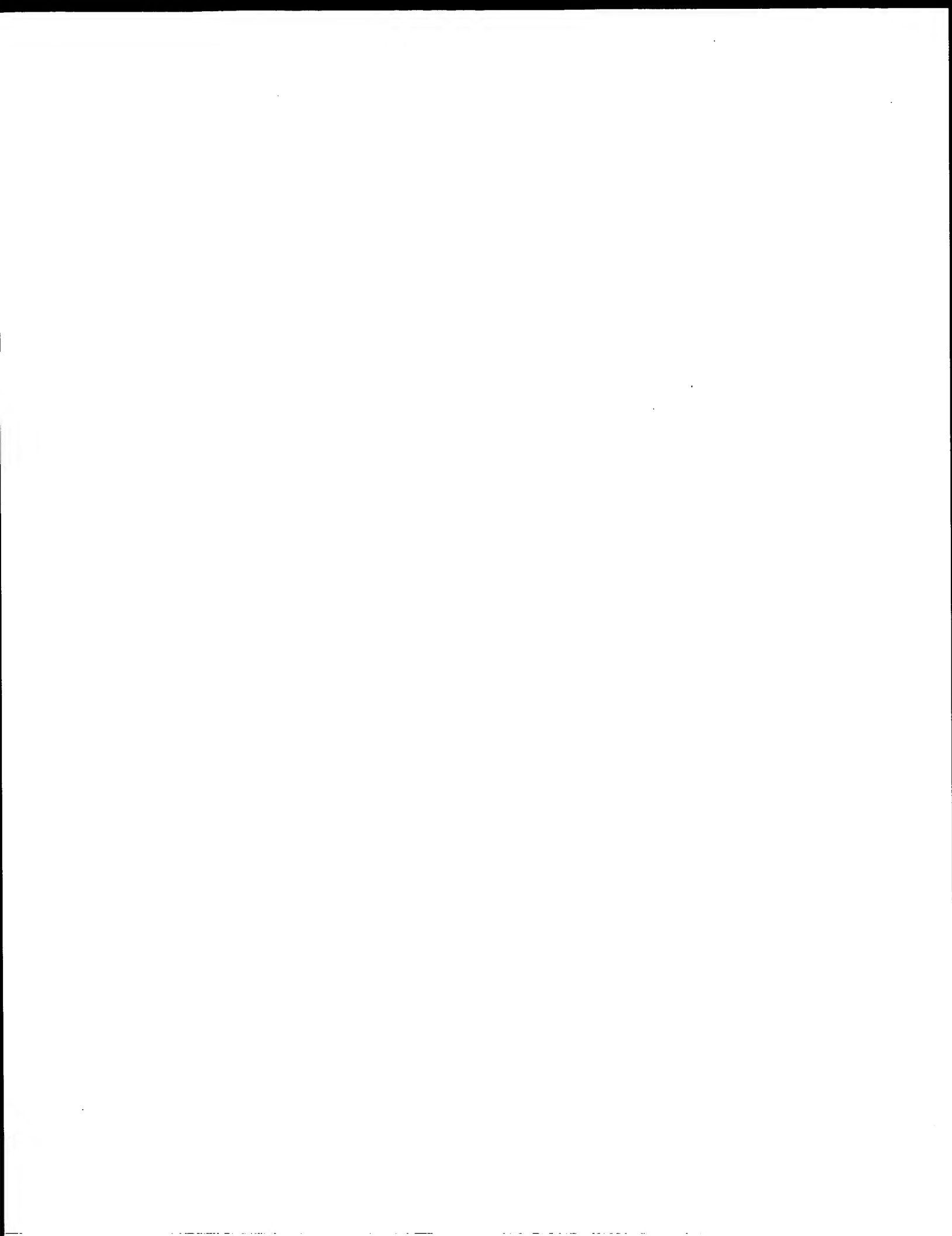


Fig. 2.1

CTGTGTCCCG CAGCCGGATA ACCTGGCTGA CCCGATTCCG CGGACACCCG TGCAGCCGCG

GCTGGAGCCA GGGCGCCGGT GCCCGCGCTC TCCCCGGTCT TGCCTGCGG GGGCCGATAC

CGCCTCTGTG ACTTCTTTGC GGGCCAGGGA CGGAGAAGGA GTCTGTGCCT GAGAACTGG

GCTCTGTGCC CAGGCGCGAG GTGCAGG ATG GAG AGC AAG GGC CTG CTA GCT
 Met Glu Ser Lys Gly Leu Leu Ala
 -19 -15

GTC GCT CTG TGG TTC TGC GTG GAG ACC CGA GCC GCC TCT GTG GGT TTG
 Val Ala Leu Trp Phe Cys Val Glu Thr Arg Ala Ala Ser Val Gly Leu
 -10 -5 1 5

CCT GGC GAT TTT CTC CAT CCC CCC AAG CTC AGC ACA CAG AAA GAC ATA
 Pro Gly Asp Phe Leu His Pro Pro Lys Leu Ser Thr Gln Lys Asp Ile
 10 15 20

CTG ACA ATT TTG GCA AAT ACA ACC CTT CAG ATT ACT TGC AGG GGA CAG
 Leu Thr Ile Leu Ala Asn Thr Thr Leu Gln Ile Thr Cys Arg Gly Gln
 25 30 35

CGG GAC CTG GAC TGG CTT TGG CCC AAT GCT CAG CGT GAT TCT GAG GAA
 Arg Asp Leu Asp Trp Leu Trp Pro Asn Ala Gln Arg Asp Ser Glu Glu
 40 45 50

AGG GTA TTG GTG ACT GAA TGC GGC GGT GGT GAC AGT ATC TTC TGC AAA
 Arg Val Leu Val Thr Glu Cys Gly Gly Gly Asp Ser Ile Phe Cys Lys
 55 60 65

ACA CTC ACC ATT CCC AGG GTG GTT GGA AAT GAT ACT GGA GCC TAC AAG
 Thr Leu Thr Ile Pro Arg Val Val Gly Asn Asp Thr Gly Ala Tyr Lys
 70 75 80 85

TGC TCG TAC CGG GAC GTC GAC ATA GCC TCC ACT GTT TAT GTC TAT GTT
 Cys Ser Tyr Arg Asp Val Asp Ile Ala Ser Thr Val Tyr Val Tyr Val
 90 95 100

CGA GAT TAC AGA TCA CCA TTC ATC GCC TCT GTC AGT GAC CAG CAT GGC
 Arg Asp Tyr Arg Ser Pro Phe Ile Ala Ser Val Ser Asp Gln His Gly
 105 110 115

ATC GTG TAC ATC ACC GAG AAC AAG AAC AAA ACT GTG GTG ATC CCC TGC
 Ile Val Tyr Ile Thr Glu Asn Lys Asn Lys Thr Val Val Ile Pro Cys
 120 125 130

Fig. 2.2

CGA Arg 135	GGG Gly	TCG Ser	ATT Ile	TCA Ser	AAC Asn 140	CTC Leu	AAT Asn	GTG Val	TCT Ser	CTT Leu	TGC Cys 145	GCT Ala	AGG Arg	TAT Tyr	CCA Pro
GAA Glu 150	AAG Lys	AGA Arg	TTT Phe	GTT Val	CCG Pro 155	GAT Asp	GGA Gly	AAC Asn	AGA Arg	ATT Ile 160	TCC Ser	TGG Trp	GAC Asp	AGC Ser	GAG Glu 165
ATA Ile	GGC Gly	TTT Phe	ACT Thr	CTC Leu 170	CCC Pro	AGT Ser	TAC Tyr	ATG Met	ATC Ile 175	AGC Ser	TAT Tyr	GCC Ala	GGC Gly	ATG Met 180	GTC Val
TTC Phe	TGT Cys	GAG Glu	GCA Ala 185	AAG Lys	ATC Ile	AAT Asn	GAT Asp	GAA Glu 190	ACC Thr	TAT Tyr	CAG Gln	TCT Ser	ATC Ile 195	ATG Met	TAC Tyr
ATA Ile	GTT Val 200	GTG Val	GTT Val	GTA Val	GGA Gly	TAT Tyr	AGG Arg 205	ATT Ile	TAT Tyr	GAT Asp	GTG Val 210	ATT Ile	CTG Leu	AGC Ser	CCC Pro
CCG Pro 215	CAT His	GAA Glu	ATT Ile	GAG Glu	CTA Leu	TCT Ser 220	GCC Ala	GGA Gly	GAA Glu	AAA Lys	CTT Leu 225	GTC Val	TTA Leu	AAT Asn	TGT Cys
ACA Thr 230	GCG Ala	AGA Arg	ACA Thr	GAG Glu	CTC Leu 235	AAT Asn	GTG Val	GGG Gly	CTT Leu	GAT Asp 240	TTC Phe	ACC Thr	TGG Trp	CAC His	TCT Ser 245
CCA Pro	CCT Pro	TCA Ser	AAG Lys	TCT Ser 250	CAT His	CAT His	AAG Lys	AAG Lys	ATT Ile 255	GTA Val	AAC Asn	CGG Arg	GAT Asp	GTG Val 260	AAA Lys
CCC Pro	TTT Phe	CCT Pro	GGG Gly 265	ACT Thr	GTG Val	GCG Ala	AAG Lys	ATG Met 270	TTT Phe	TTG Leu	AGC Ser	ACC Thr	TTG Leu 275	ACA Thr	ATA Ile
GAA Glu	AGT Ser 280	GTG Val	ACC Thr	AAG Lys	AGT Ser	GAC Asp	CAA Gln 285	GGG Gly	GAA Glu	TAC Tyr	ACC Thr	TGT Cys 290	GTA Val	GCG Ala	TCC Ser
AGT Ser 295	GGA Gly	CGG Arg	ATG Met	ATC Ile	AAG Lys	AGA Arg 300	AAT Asn	AGA Arg	ACA Thr	TTT Phe	GTC Val 305	CGA Arg	GTT Val	CAC His	ACA Thr
AAG Lys 310	CCT Pro	TTT Phe	ATT Ile	GCT Ala	TTC Phe 315	GGT Gly	AGT Ser	GGG Gly	ATG Met	AAA Lys 320	TCT Ser	TTG Leu	GTG Val	GAA Glu	GCC Ala 325

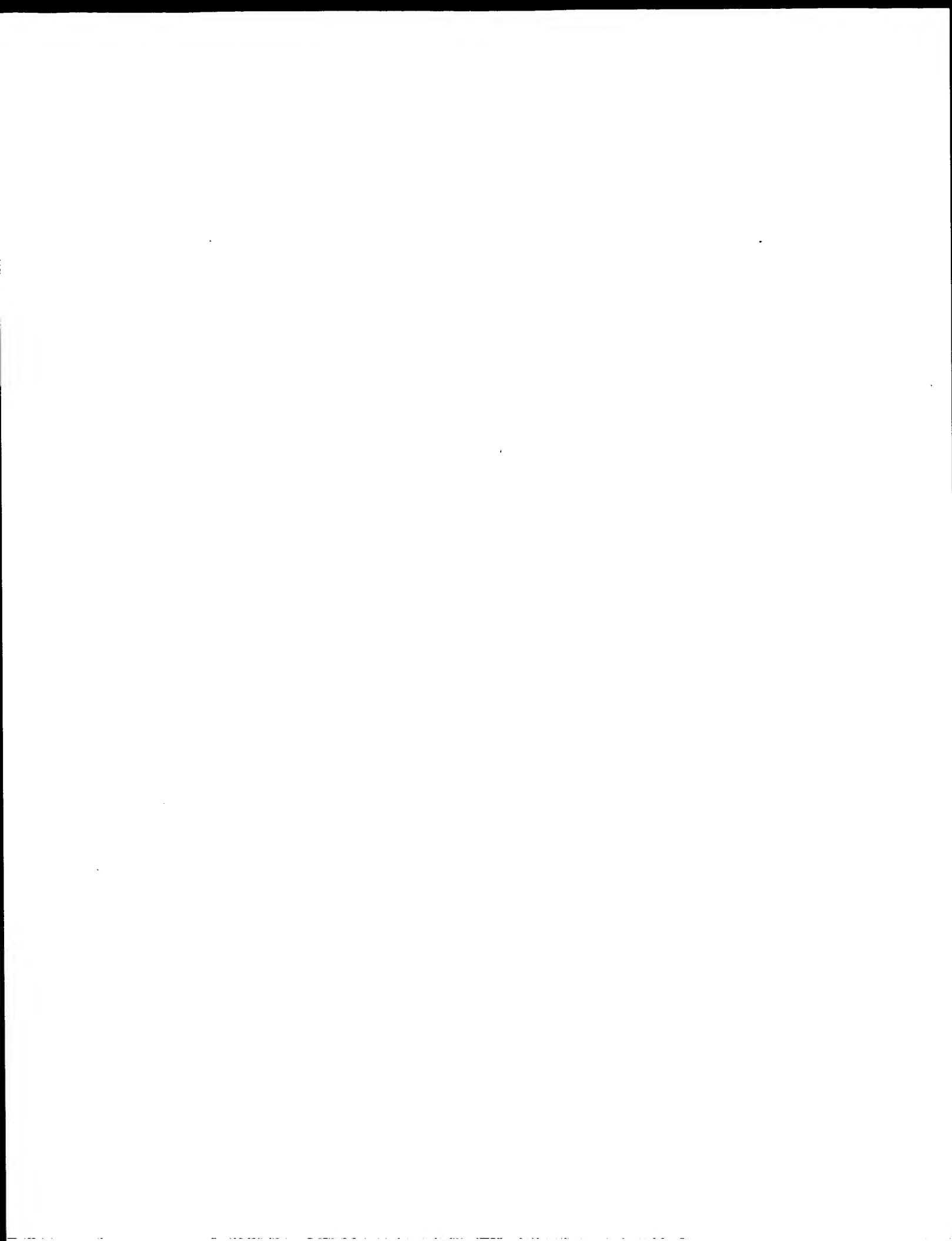


Fig. 2.3

ACA Thr	GTG Val	GGC Gly	AGT Ser	CAA Gln 330	GTC Val	CGA Arg	ATC Ile	CCT Pro	GTG Val 335	AAG Lys	TAT Tyr	CTC Leu	AGT Ser	TAC Tyr 340	CCA Pro
GCT Ala	CCT Pro	GAT Asp	ATC Ile 345	AAA Lys	TGG Trp	TAC Tyr	AGA Arg	AAT Asn 350	GGA Gly	AGG Arg	CCC Pro	ATT Ile	GAG Glu 355	TCC Ser	AAC Asn
TAC Tyr	ACA Thr	ATG Met 360	ATT Ile	GTT Val	GGC Gly	GAT Asp	GAA Glu 365	CTC Leu	ACC Thr	ATC Ile	ATG Met	GAA Glu 370	GTG Val	ACT Thr	GAA Glu
AGA Arg	GAT Asp 375	GCA Ala	GGA Gly	AAC Asn	TAC Tyr	ACG Thr 380	GTC Val	ATC Ile	CTC Leu	ACC Thr	AAC Asn 385	CCC Pro	ATT Ile	TCA Ser	ATG Met
GAG Glu 390	AAA Lys	CAG Gln	AGC Ser	CAC His	ATG Met 395	GTC Val	TCT Ser	CTG Leu	GTT Val	GTG Val 400	AAT Asn	GTC Val	CCA Pro	CCC Pro	CAG Gln 405
ATC Ile	GGT Gly	GAG Glu	AAA Lys	GCC Ala 410	TTG Leu	ATC Ile	TCG Ser	CCT Pro	ATG Met 415	GAT Asp	TCC Ser	TAC Tyr	CAG Gln	TAT Tyr 420	GGG Gly
ACC Thr	ATG Met	CAG Gln	ACA Thr 425	TTG Leu	ACA Thr	TGC Cys	ACA Thr	GTC Val 430	TAC Tyr	GCC Ala	AAC Asn	CCT Pro	CCC Pro 435	CTG Leu	CAC His
CAC His	ATC Ile	CAG Gln 440	TGG Trp	TAC Tyr	TGG Trp	CAG Gln	CTA Leu 445	GAA Glu	GAA Glu	GCC Ala	TGC Cys	TCC Ser 450	TAC Tyr	AGA Arg	CCC Pro
GGC Gly	CAA Gln 455	ACA Thr	AGC Ser	CCG Pro	TAT Tyr	GCT Ala 460	TGT Cys	AAA Lys	GAA Glu	TGG Trp	AGA Arg 465	CAC His	GTG Val	GAG Glu	GAT Asp
TTC Phe 470	CAG Gln	GGG Gly	GGA Gly	AAC Asn	AAG Lys 475	ATC Ile	GAA Glu	GTC Val	ACC Thr	AAA Lys 480	AAC Asn	CAA Gln	TAT Tyr	GCC Ala	CTG Leu 485
ATT Ile	GAA Glu	GGA Gly	AAA Lys	AAC Asn 490	AAA Lys	ACT Thr	GTA Val	AGT Ser	ACG Thr 495	CTG Leu	GTC Val	ATC Ile	CAA Gln	GCT Ala 500	GCC Ala
AAC Asn	GTG Val	TCA Ser	GCG Ala 505	TTG Leu	TAC Tyr	AAA Lys	TGT Cys	GAA Glu 510	GCC Ala	ATC Ile	AAC Asn	AAA Lys	GCG Ala 515	GGA Gly	CGA Arg

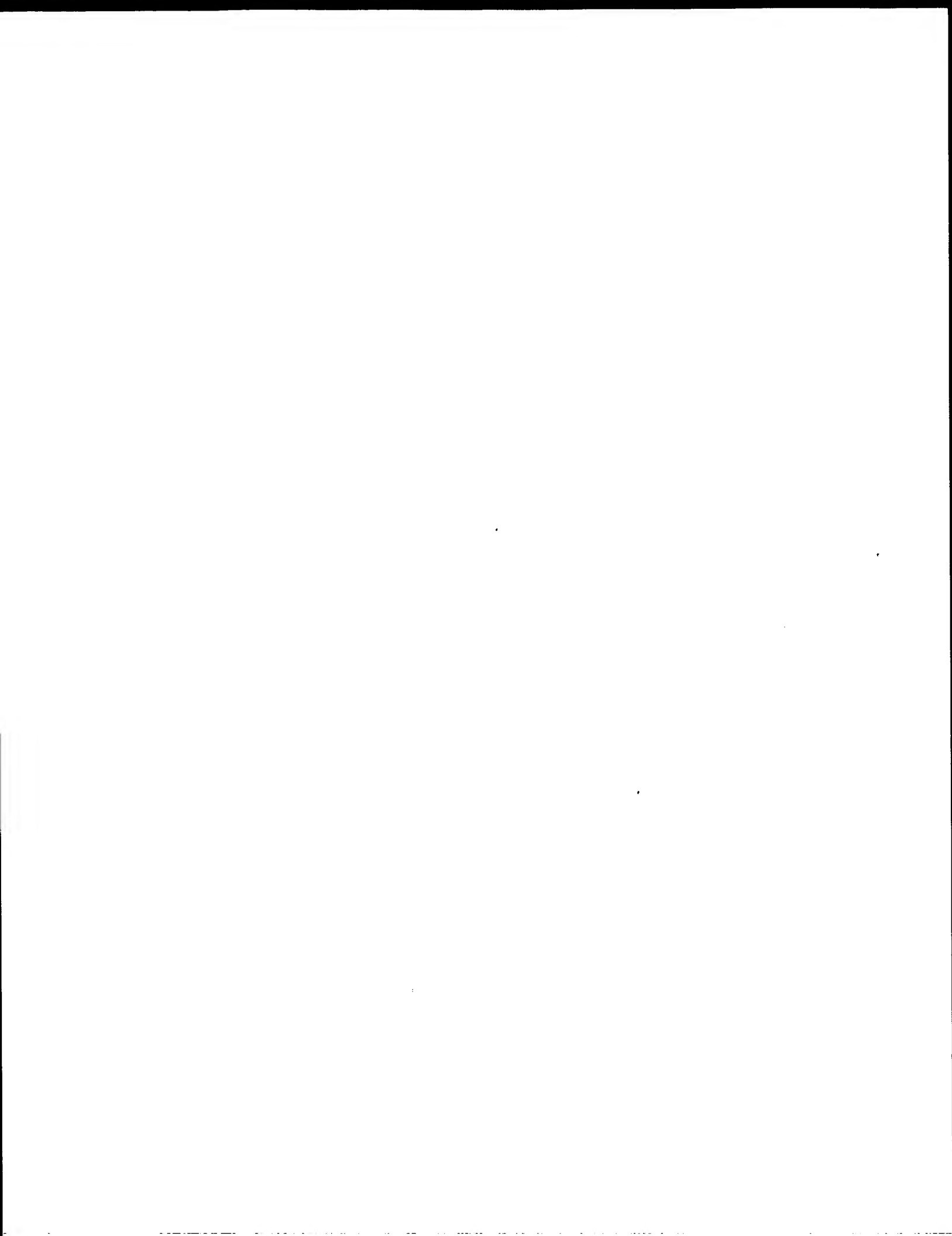


Fig. 2.4

GGA	GAG	AGG	GTC	ATC	TCC	TTC	CAT	GTG	ATC	AGG	GGT	CCT	GAA	ATT	ACT
Gly	Glu	Arg	Val	Ile	Ser	Phe	His	Val	Ile	Arg	Gly	Pro	Glu	Ile	Thr
		520					525					530			
GTG	CAA	CCT	GCT	GCC	CAG	CCA	ACT	GAG	CAG	GAG	AGT	GTG	TCC	CTG	TTG
Val	Gln	Pro	Ala	Ala	Gln	Pro	Thr	Glu	Gln	Glu	Ser	Val	Ser	Leu	Leu
	535					540					545				
TGC	ACT	GCA	GAC	AGA	AAT	ACG	TTT	GAG	AAC	CTC	ACG	TGG	TAC	AAG	CTT
Cys	Thr	Ala	Asp	Arg	Asn	Thr	Phe	Glu	Asn	Leu	Thr	Trp	Tyr	Lys	Leu
550					555					560					565
GGC	TCA	CAG	GCA	ACA	TCG	GTC	CAC	ATG	GGC	GAA	TCA	CTC	ACA	CCA	GTT
Gly	Ser	Gln	Ala	Thr	Ser	Val	His	Met	Gly	Glu	Ser	Leu	Thr	Pro	Val
				570					575					580	
TGC	AAG	AAC	TTG	GAT	GCT	CTT	TGG	AAA	CTG	AAT	GGC	ACC	ATG	TTT	TCT
Cys	Lys	Asn	Leu	Asp	Ala	Leu	Trp	Lys	Leu	Asn	Gly	Thr	Met	Phe	Ser
			585					590					595		
AAC	AGC	ACA	AAT	GAC	ATC	TTG	ATT	GTG	GCA	TTT	CAG	AAT	GCC	TCT	CTG
Asn	Ser	Thr	Asn	Asp	Ile	Leu	Ile	Val	Ala	Phe	Gln	Asn	Ala	Ser	Leu
		600					605					610			
CAG	GAC	CAA	GGC	GAC	TAT	GTT	TGC	TCT	GCT	CAA	GAT	AAG	AAG	ACC	AAG
Gln	Asp	Gln	Gly	Asp	Tyr	Val	Cys	Ser	Ala	Gln	Asp	Lys	Lys	Thr	Lys
	615					620					625				
AAA	AGA	CAT	TGC	CTG	GTC	AAA	CAG	CTC	ATC	ATC	CTA	GAG	CGC	ATG	GCA
Lys	Arg	His	Cys	Leu	Val	Lys	Gln	Leu	Ile	Ile	Leu	Glu	Arg	Met	Ala
630					635					640					645
CCC	ATG	ATC	ACC	GGA	AAT	CTG	GAG	AAT	CAG	ACA	ACA	ACC	ATT	GGC	GAG
Pro	Met	Ile	Thr	Gly	Asn	Leu	Glu	Asn	Gln	Thr	Thr	Thr	Ile	Gly	Glu
				650					655					660	
ACC	ATT	GAA	GTG	ACT	TGC	CCA	GCA	TCT	GGA	AAT	CCT	ACC	CCA	CAC	ATT
Thr	Ile	Glu	Val	Thr	Cys	Pro	Ala	Ser	Gly	Asn	Pro	Thr	Pro	His	Ile
			665					670					675		
ACA	TGG	TTC	AAA	GAC	AAC	GAG	ACC	CTG	GTA	GAA	GAT	TCA	GGC	ATT	GTA
Thr	Trp	Phe	Lys	Asp	Asn	Glu	Thr	Leu	Val	Glu	Asp	Ser	Gly	Ile	Val
		680					685					690			
CTG	AGA	GAT	GGG	AAC	CGG	AAC	CTG	ACT	ATC	CGC	AGG	GTG	AGG	AAG	GAG
Leu	Arg	Asp	Gly	Asn	Arg	Asn	Leu	Thr	Ile	Arg	Arg	Val	Arg	Lys	Glu
	695					700					705				

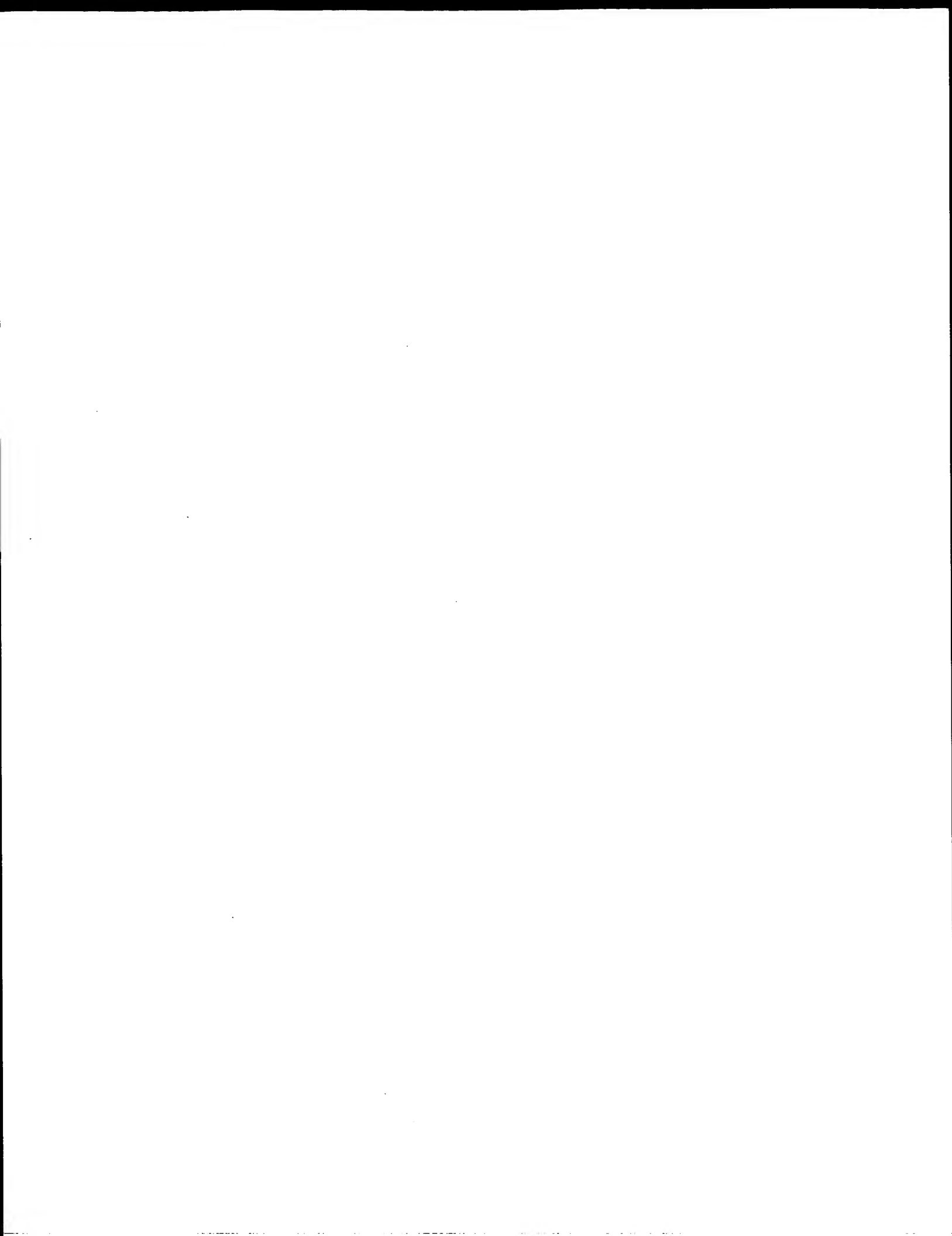


Fig. 2.5

GAT Asp 710	GGA Gly	GGC Gly	CTC Leu	TAC Tyr	ACC Thr 715	TGC Cys	CAG Gln	GCC Ala	TGC Cys	AAT Asn 720	GTC Val	CTT Leu	GGC Gly	TGT Cys	GCA Ala 725
AGA Arg	GCG Ala	GAG Glu	ACG Thr	CTC Leu 730	TTC Phe	ATA Ile	ATA Ile	GAA Glu	GGT Gly 735	GCC Ala	CAG Gln	GAA Glu	AAG Lys	ACC Thr 740	AAC Asn
TTG Leu	GAA Glu	GTC Val	ATT Ile 745	ATC Ile	CTC Leu	GTC Val	GGC Gly	ACT Thr 750	GCA Ala	GTG Val	ATT Ile	GCC Ala	ATG Met 755	TTC Phe	TTC Phe
TGG Trp	CTC Leu	CTT Leu 760	CTT Leu	GTC Val	ATT Ile	CTC Leu	GTA Val 765	CGG Arg	ACC Thr	GTT Val	AAG Lys	CGG Arg 770	GCC Ala	AAT Asn	GAA Glu
GGG Gly 775	GAA Glu	CTG Leu	AAG Lys	ACA Thr	GGC Gly	TAC Tyr 780	TTG Leu	TCT Ser	ATT Ile	GTC Val	ATG Met 785	GAT Asp	CCA Pro	GAT Asp	GAA Glu
TTG Leu 790	CCC Pro	TTG Leu	GAT Asp	GAG Glu	CGC Arg 795	TGT Cys	GAA Glu	CGC Arg	TTG Leu	CCT Pro 800	TAT Tyr	GAT Asp	GCC Ala	AGC Ser	AAG Lys 805
TGG Trp	GAA Glu	TTC Phe	CCC Pro	AGG Arg 810	GAC Asp	CGG Arg	CTG Leu	AAA Lys	CTA Leu 815	GGA Gly	AAA Lys	CCT Pro	CTT Leu	GGC Gly 820	CGC Arg
GGT Gly	GCC Ala	TTC Phe	GGC Gly 825	CAA Gln	GTG Val	ATT Ile	GAG Glu	GCA Ala 830	GAC Asp	GCT Ala	TTT Phe	GGA Gly	ATT Ile 835	GAC Asp	AAG Lys
ACA Thr	GCG Ala	ACT Thr 840	TGC Cys	AAA Lys	ACA Thr	GTA Val	GCC Ala 845	GTC Val	AAG Lys	ATG Met	TTG Leu	AAA Lys 850	GAA Glu	GGA Gly	GCA Ala
ACA Thr 855	CAC His	AGC Ser	GAG Glu	CAT His	CGA Arg	GCC Ala 860	CTC Leu	ATG Met	TCT Ser	GAA Glu	CTC Leu 865	AAG Lys	ATC Ile	CTC Leu	ATC Ile
CAC His 870	ATT Ile	GGT Gly	CAC His	CAT His	CTC Leu 875	AAT Asn	GTG Val	GTG Val	AAC Asn	CTC Leu 880	CTA Leu	GGC Gly	GCC Ala	TGC Cys	ACC Thr 885
AAG Lys	CCG Pro	GGA Gly	GGG Gly	CCT Pro 890	CTC Leu	ATG Met	GTG Val	ATT Ile	GTG Val 895	GAA Glu	TTC Phe	TCG Ser	AAG Lys	TTT Phe 900	GGA Gly

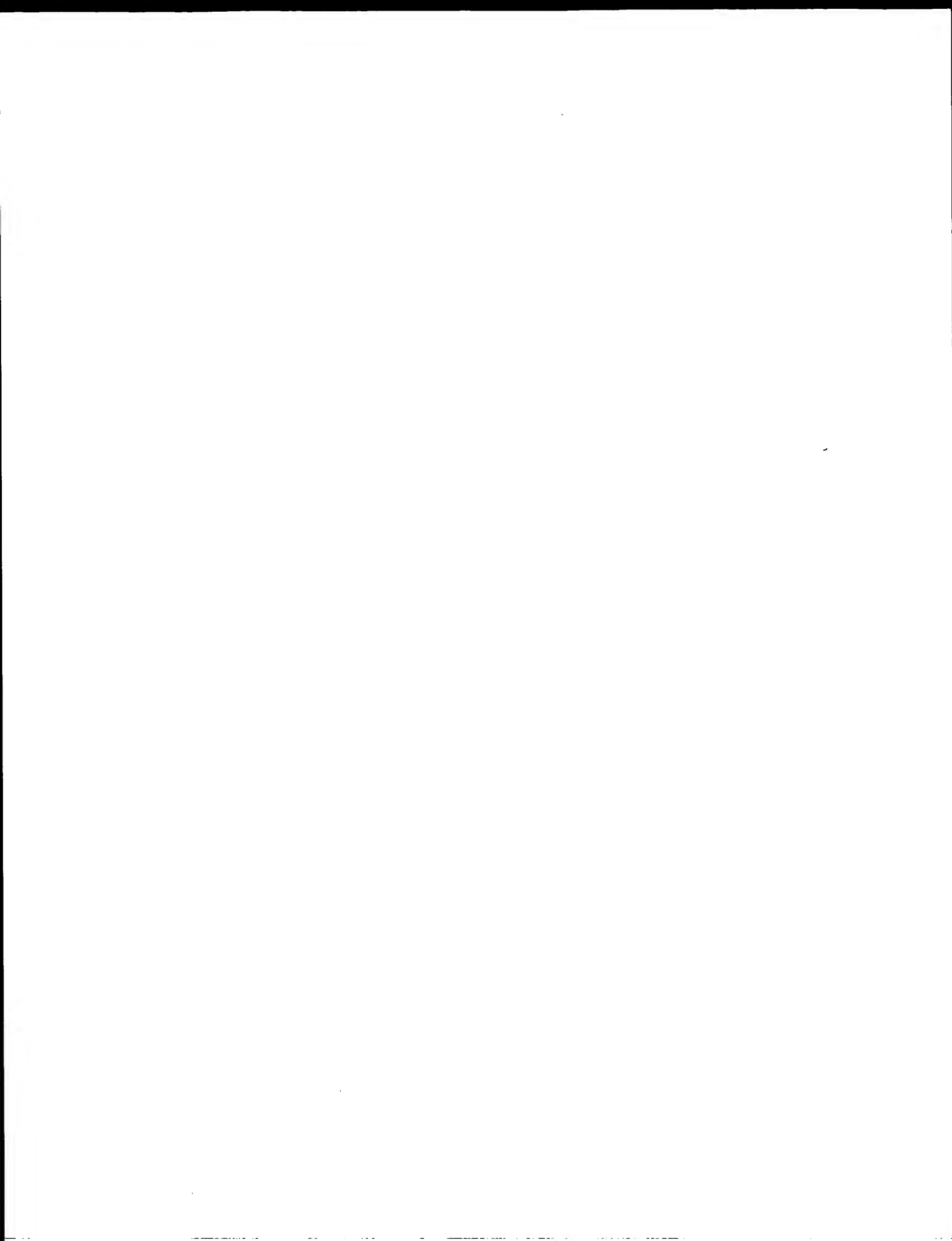


Fig. 2.6

AAC	CTA	TCA	ACT	TAC	TTA	CGG	GGC	AAG	AGA	AAT	GAA	TTT	GTT	CCC	TAT	
Asn	Leu	Ser	Thr	Tyr	Leu	Arg	Gly	Lys	Arg	Asn	Glu	Phe	Val	Pro	Tyr	
			905					910					915			
AAG	AGC	AAA	GGG	GCA	CGC	TTC	CGC	CAG	GGC	AAG	GAC	TAC	GTT	GGG	GAG	
Lys	Ser	Lys	Gly	Ala	Arg	Phe	Arg	Gln	Gly	Lys	Asp	Tyr	Val	Gly	Glu	
		920					925					930				
CTC	TCC	GTG	GAT	CTG	AAA	AGA	CGC	TTG	GAC	AGC	ATC	ACC	AGC	AGC	CAG	
Leu	Ser	Val	Asp	Leu	Lys	Arg	Arg	Leu	Asp	Ser	Ile	Thr	Ser	Ser	Gln	
	935					940					945					
AGC	TCT	GCC	AGC	TCA	GGC	TTT	GTT	GAG	GAG	AAA	TCG	CTC	AGT	GAT	GTA	
Ser	Ser	Ala	Ser	Ser	Gly	Phe	Val	Glu	Glu	Lys	Ser	Leu	Ser	Asp	Val	
950					955					960					965	
GAG	GAA	GAA	GAA	GCT	TCT	GAA	GAA	CTG	TAC	AAG	GAC	TTC	CTG	ACC	TTG	
Glu	Glu	Glu	Glu	Ala	Ser	Glu	Glu	Leu	Tyr	Lys	Asp	Phe	Leu	Thr	Leu	
				970					975					980		
GAG	CAT	CTC	ATC	TGT	TAC	AGC	TTC	CAA	GTG	GCT	AAG	GGC	ATG	GAG	TTC	
Glu	His	Leu	Ile	Cys	Tyr	Ser	Phe	Gln	Val	Ala	Lys	Gly	Met	Glu	Phe	
			985					990					995			
TTG	GCA	TCA	AGG	AAG	TGT	ATC	CAC	AGG	GAC	CTG	GCA	GCA	CGA	AAC	ATT	
Leu	Ala	Ser	Arg	Lys	Cys	Ile	His	Arg	Asp	Leu	Ala	Ala	Arg	Asn	Ile	
		1000					1005					1010				
CTC	CTA	TCG	GAG	AAG	AAT	GTG	GTT	AAG	ATC	TGT	GAC	TTC	GGC	TTG	GCC	
Leu	Leu	Ser	Glu	Lys	Asn	Val	Val	Lys	Ile	Cys	Asp	Phe	Gly	Leu	Ala	
		1015				1020					1025					
CGG	GAC	ATT	TAT	AAA	GAC	CCG	GAT	TAT	GTC	AGA	AAA	GGA	GAT	GCC	CGA	
Arg	Asp	Ile	Tyr	Lys	Asp	Pro	Asp	Tyr	Val	Arg	Lys	Gly	Asp	Ala	Arg	
1030					1035					1040					1045	
CTC	CCT	TTG	AAG	TGG	ATG	GCC	CCG	GAA	ACC	ATT	TTT	GAC	AGA	GTA	TAC	
Leu	Pro	Leu	Lys	Trp	Met	Ala	Pro	Glu	Thr	Ile	Phe	Asp	Arg	Val	Tyr	
			1050						1055					1060		
ACA	ATT	CAG	AGC	GAT	GTG	TGG	TCT	TTC	GGT	GTG	TTG	CTC	TGG	GAA	ATA	
Thr	Ile	Gln	Ser	Asp	Val	Trp	Ser	Phe	Gly	Val	Leu	Leu	Trp	Glu	Ile	
		1065						1070					1075			
TTT	TCC	TTA	GGT	GCC	TCC	CCA	TAC	CCT	GGG	GTC	AAG	ATT	GAT	GAA	GAA	
Phe	Ser	Leu	Gly	Ala	Ser	Pro	Tyr	Pro	Gly	Val	Lys	Ile	Asp	Glu	Glu	
		1080					1085					1090				

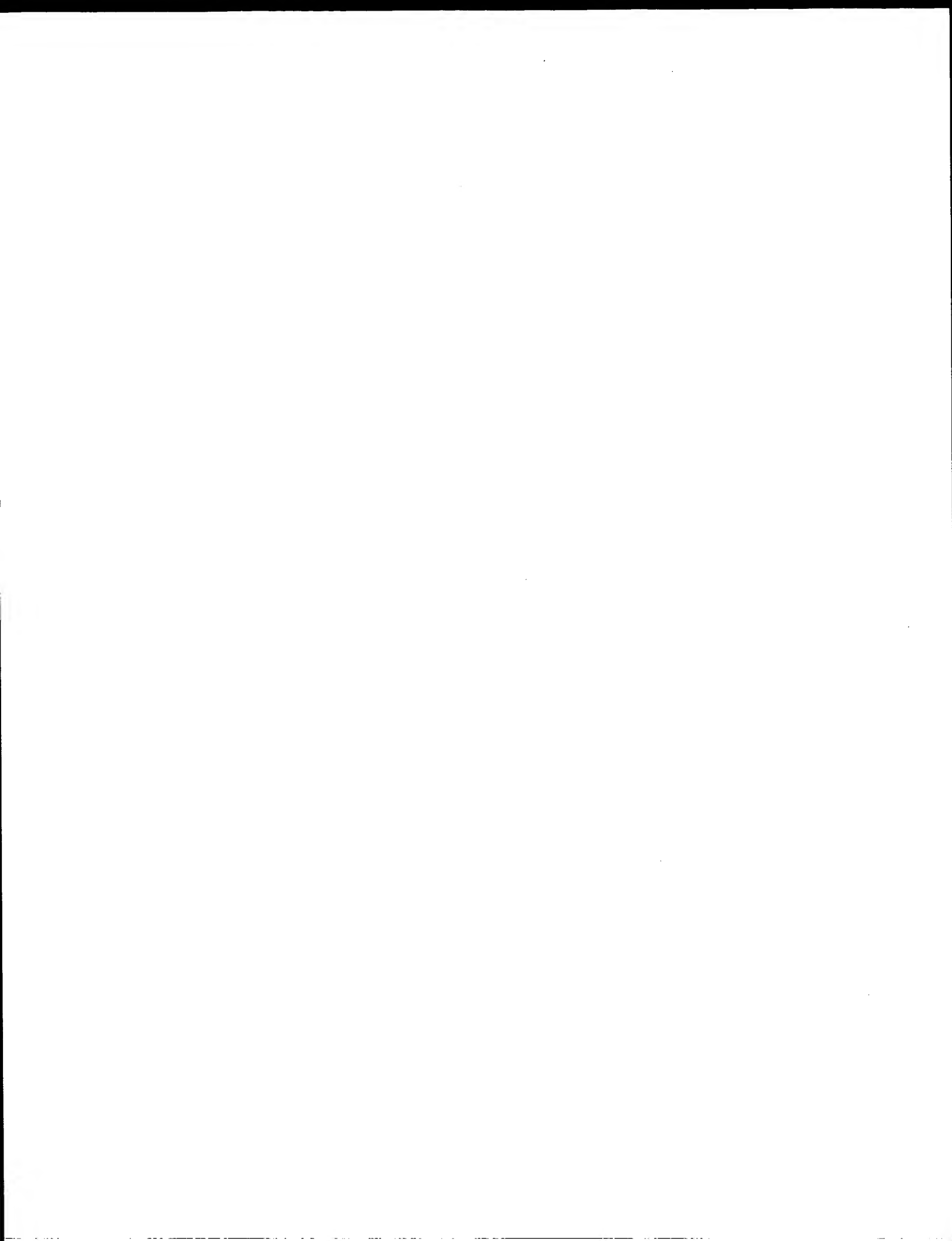


Fig. 2.7

TTT Phe 1095	TGT Cys 1095	AGG Arg 1095	AGA Arg 1095	TTG Leu 1095	AAA Lys 1100	GAA Glu 1100	GGA Gly 1100	ACT Thr 1100	AGA Arg 1100	ATG Met 1105	CGG Arg 1105	GCT Ala 1105	CCT Pro 1105	GAC Asp 1105	TAC Tyr 1105
ACT Thr 1110	ACC Thr 1110	CCA Pro 1110	GAA Glu 1110	ATG Met 1110	TAC Tyr 1115	CAG Gln 1115	ACC Thr 1115	ATG Met 1115	CTG Leu 1120	GAC Asp 1120	TGC Cys 1120	TGG Trp 1120	CAT His 1125	GAG Glu 1125	GAC Asp 1125
CCC Pro 1130	AAC Asn 1130	CAG Gln 1130	AGA Arg 1130	CCC Pro 1130	TCG Ser 1130	TTT Phe 1135	TCA Ser 1135	GAG Glu 1135	TTG Leu 1135	GTG Val 1135	GAG Glu 1140	CAT His 1140	TTG Leu 1140	GGA Gly 1140	AAC Asn 1140
CTC Leu 1145	CTG Leu 1145	CAA Gln 1145	GCA Ala 1145	AAT Asn 1145	GCG Ala 1145	CAG Gln 1150	CAG Gln 1150	GAT Asp 1150	GGC Gly 1150	AAA Lys 1150	GAC Asp 1155	TAT Tyr 1155	ATT Ile 1155	GTT Val 1155	CTT Leu 1155
CCA Pro 1160	ATG Met 1160	TCA Ser 1160	GAG Glu 1160	ACA Thr 1160	CTG Leu 1165	AGC Ser 1165	ATG Met 1165	GAA Glu 1165	GAG Glu 1170	GAT Asp 1170	TCT Ser 1170	GGA Gly 1170	CTC Leu 1170	TCC Ser 1170	CTG Leu 1170
CCT Pro 1175	ACC Thr 1175	TCA Ser 1175	CCT Pro 1175	GTT Val 1180	TCC Ser 1180	TGT Cys 1180	ATG Met 1180	GAG Glu 1185	GAA Glu 1185	GAG Glu 1185	GAA Glu 1185	GTG Val 1185	TGC Cys 1185	GAC Asp 1185	CCC Pro 1185
AAA Lys 1190	TTC Phe 1190	CAT His 1190	TAT Tyr 1190	GAC Asp 1195	AAC Asn 1195	ACA Thr 1195	GCA Ala 1200	GGA Gly 1200	ATC Ile 1200	AGT Ser 1200	CAT His 1205	TAT Tyr 1205	CTC Leu 1205	CAG Gln 1205	AAC Asn 1205
AGT Ser 1210	AAG Lys 1210	CGA Arg 1210	AAG Lys 1210	AGC Ser 1210	CGG Arg 1215	CCA Pro 1215	GTG Val 1215	AGT Ser 1215	GTA Val 1215	AAA Lys 1220	ACA Thr 1220	TTT Phe 1220	GAA Glu 1220	GAT Asp 1220	ATC Ile 1220
CCA Pro 1225	TTG Leu 1225	GAG Glu 1225	GAA Glu 1225	CCA Pro 1225	GAA Glu 1230	GTA Val 1230	AAA Lys 1230	GTG Val 1230	ATC Ile 1230	CCA Pro 1235	GAT Asp 1235	GAC Asp 1235	AGC Ser 1235	CAG Gln 1235	ACA Thr 1235
GAC Asp 1240	AGT Ser 1240	GGG Gly 1240	ATG Met 1240	GTC Val 1245	CTT Leu 1245	GCA Ala 1245	TCA Ser 1245	GAA Glu 1250	GAG Glu 1250	CTG Leu 1250	AAA Lys 1250	ACT Thr 1250	CTG Leu 1250	GAA Glu 1250	GAC Asp 1250
AGG Arg 1255	AAC Asn 1255	AAA Lys 1255	TTA Leu 1255	TCT Ser 1260	CCA Pro 1260	TCT Ser 1260	TTT Phe 1265	GGT Gly 1265	GGA Gly 1265	ATG Met 1265	ATG Met 1265	CCC Pro 1265	AGT Ser 1265	AAA Lys 1265	AGC Ser 1265
AGG Arg 1270	GAG Glu 1270	TCT Ser 1270	GTG Val 1270	GCC Ala 1275	TCG Ser 1275	GAA Glu 1275	GGC Gly 1275	TCC Ser 1280	AAC Asn 1280	CAG Gln 1280	ACC Thr 1280	AGT Ser 1285	GGC Gly 1285	TAC Tyr 1285	CAG Gln 1285

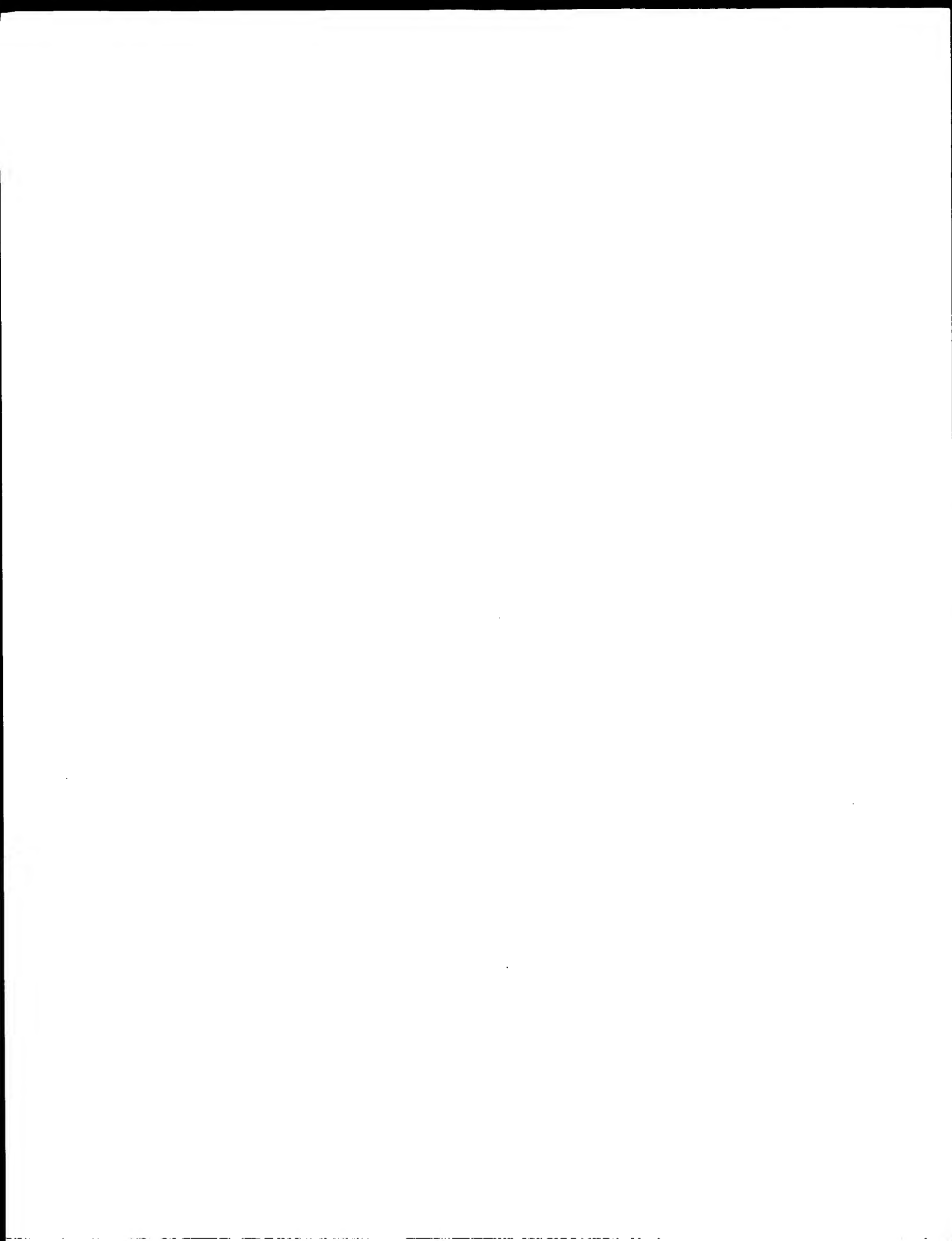
Fig. 2.8

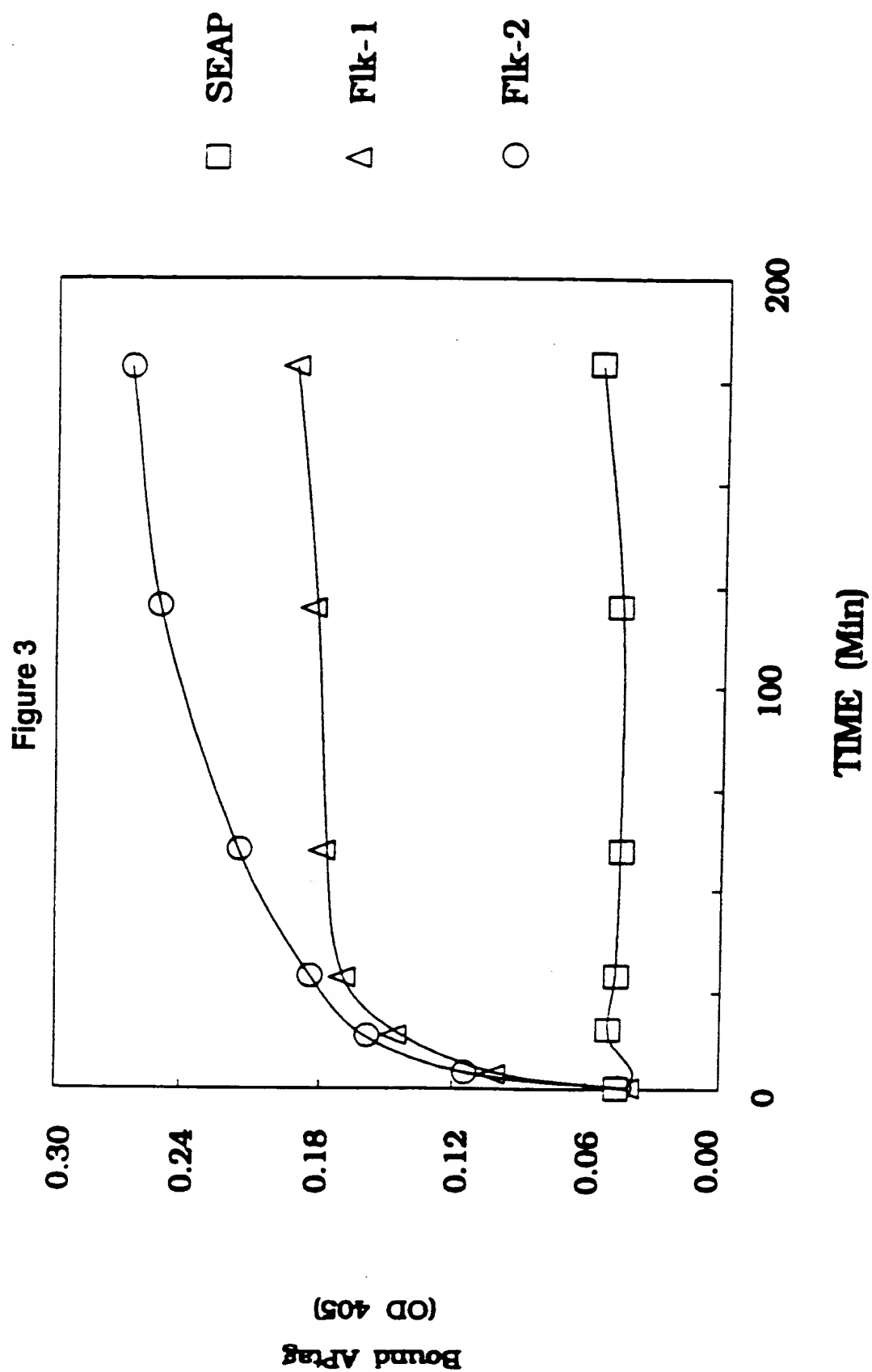
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Ser	Gly	Tyr	His	Ser	Asp	Asp	Thr	Asp	Thr	Thr	Val	Tyr	Ser	Ser	Asp
				1290					1295					1300	
GAG	GCA	GGA	CTT	TTA	AAG	ATG	GTG	GAT	GCT	GCA	GTT	CAC	GCT	GAC	TCA
Glu	Ala	Gly	Leu	Leu	Lys	Met	Val	Asp	Ala	Ala	Val	His	Ala	Asp	Ser
			1305					1310					1315		
GGG	ACC	ACA	CTG	CAG	CTC	ACC	TCC	TGT	TTA	AAT	GGA	AGT	GGT	CCT	GTC
Gly	Thr	Thr	Leu	Gln	Leu	Thr	Ser	Cys	Leu	Asn	Gly	Ser	Gly	Pro	Val
			1320				1325					1330			
CCG	GCT	CCG	CCC	CCA	ACT	CCT	GGA	AAT	CAC	GAG	AGA	GGT	GCT	GCT	TAG
Pro	Ala	Pro	Pro	Pro	Thr	Pro	Gly	Asn	His	Glu	Arg	Gly	Ala	Ala	
			1335			1340					1345				

ATTTTCAAGT GTTGTTCTTT CCACCACCCG GAAGTAGCCA CATTTGATTT TCATTTTTTG
 AGGAGGGACC TCAGACTGCA AGGAGCTTGT CCTCAGGGCA TTTCCAGAGA AGATGCCCAT
 GACCCAAGAA TGTGTTGACT CTACTCTCTT TTCCATTCAT TTAAAAGTCC TATATAATGT
 GCCCTGCTGT GGTCTCACTA CCAGTTAAAG CAAAAGACTT TCAAACACGT GGACTCTGTC
 CTCCAAGAAG TGGCAACGGC ACCTCTGTGA AACTGGATCG AATGGGCAAT GCTTTGTGTG
 TTGAGGATGG GTGAGATGTC CCAGGGCCGA GTCTGTCTAC CTTGGAGGCT TTGTGGAGGA
 TGCGGCTATG AGCCAAGTGT TAAGTGTGGG ATGTGGACTG GGAGGAAGGA AGGCGCAAGC
 CGTCCGAGA GCGGTTGGAG CCTGCAGATG CATTGTGCTG GCTCTGGTGG AGGTGGGCTT
 GTGGCCTGTC AGGAAACGCA AAGGCGGCCG GCAGGGTTTG GTTTTGGAAG GTTTGCGTGC
 TCTTCACAGT CGGGTTACAG GCGAGTTCCC TGTGGCGTTT CCTACTCCTA ATGAGAGTTC
 CTTCCGGACT CTTACGTGTC TCCTGGCCTG GCCCCAGGAA GGAAATGATG CAGCTTGCTC
 CTTCTCATC TCTCAGGCTG TGCCTTAATT CAGAACACCA AAAGAGAGGA ACGTCGGCAG
 AGGCTCCTGA CGGGGCCGAA GAATTGTGAG AACAGAACAG AAATCAGGG TTTCTGCTGG
 GTGGAGACCC ACGTGGCGCC CTGGTGGCAG GTCTGAGGGT TCTCTGTCAA GTGGCGGTAA
 AGGCTCAGGC TGGTGTCTT CCTCTATCTC CACTCCTGTC AGGCCCCCAA GTCCTCAGTA
 TTTTAGCTTT GTGGCTTCCT GATGGCAGAA AAATCTTAAT TGGTTGGTTT GCTCTCCAGA

Fig. 2.9

TAATCACTAG CCAGATTTTCG AAATTACTTT TTAGCCGAGG TTATGATAAC ATCTACTGTA
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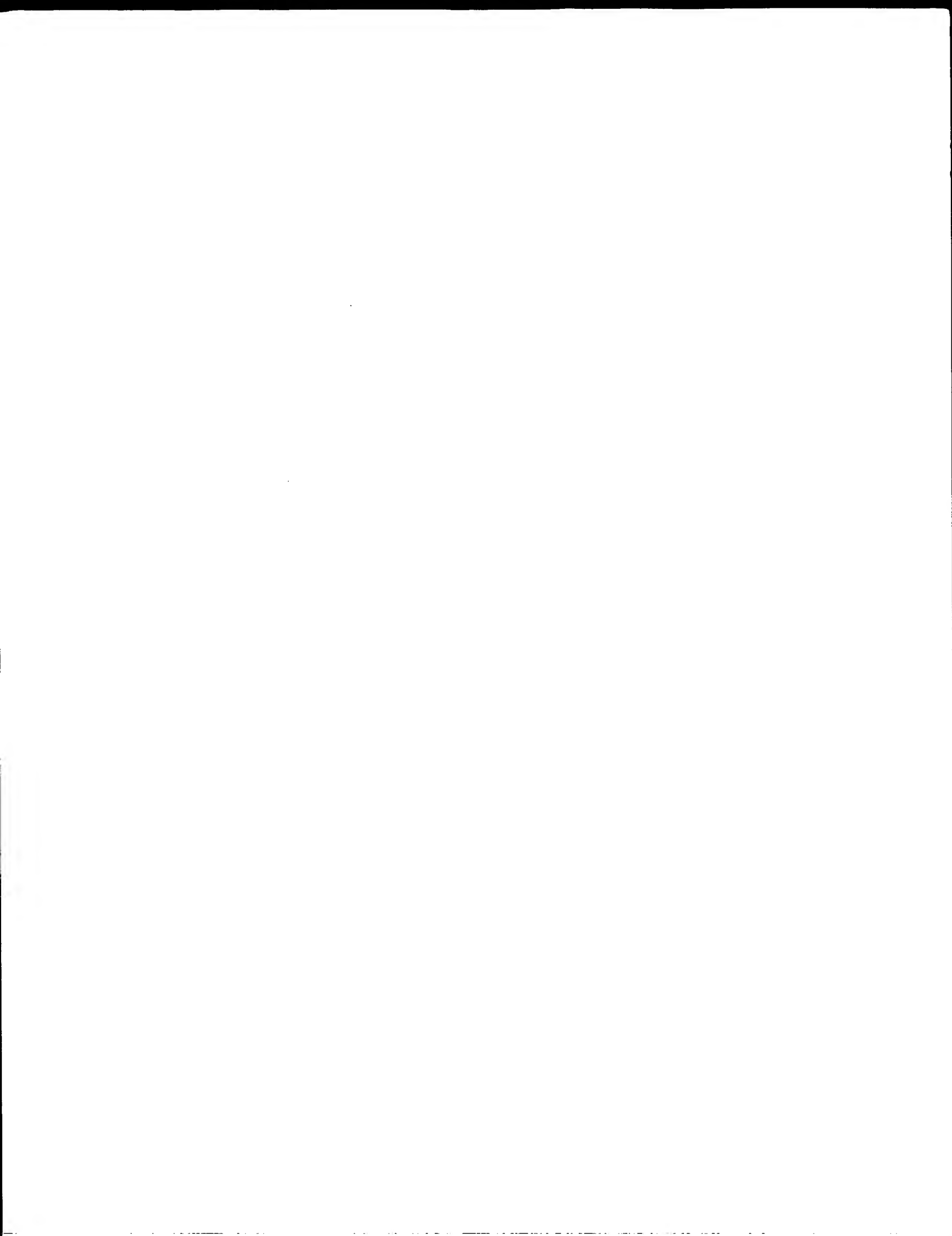


Figure 4

